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# THE JOURNAL OF AGRICULTURAL SCIENCE

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## A PRELIMINARY INVESTIGATION INTO THE OCCURRENCE OF DIFFERENT KINDS OF CARBONATES IN CERTAIN SOILS.

By F. HARDY, M.A.

(*School of Agriculture, Cambridge.*)

DURING July of 1919, the writer assisted in a soil survey of parts of the N.W. coastal belt of the Wash, which was performed at the request of the Land Reclamation Branch of the Ministry of Agriculture. The investigation outlined in the following account is an outcome of a mineralogical examination of certain of the marine silt samples then collected with a view to explaining their high carbonate content. Amongst the minerals identified in these silts was dolomite. It is believed that the presence of this mineral in the Fenland silts, and in the soils derived from them, may have some agricultural importance, for dolomite is a mineral carbonate which is little affected by weak acids, and it seems reasonable to suppose that its behaviour towards those weak acids which are liberated during certain soil processes may not be the same as that of calcium carbonate. As far as the writer is aware, the subject has received little attention from soil chemists.

Of the naturally occurring carbonates, the various forms of calcium carbonate (calcite, aragonite, etc.) are more or less easily decomposed by weak acids. Magnesite ( $\text{MgCO}_3$ ), dolomite ( $\text{CaCO}_3 \cdot \text{MgCO}_3$ ), siderite ( $\text{FeCO}_3$ ), and minerals which are complexes of these three definite compounds (such as ankerite ( $\text{CaCO}_3 \cdot (\text{Mg, Fe}) \text{CO}_3$ ), mesitite ( $2\text{MgCO}_3 \cdot \text{FeCO}_3$ ) and breunnerite ( $2\text{Mg, Fe} \text{CO}_3$ )), require a strong acid for their decomposition. Even then certain of them only react with measurable velocity when the acid is warmed.

For the sake of convenience of nomenclature, the easily decomposed carbonate minerals may be classed together under the term "calcitoid carbonates," and those more difficult to decompose by acids may be designated "dolomitoid carbonates."

In the estimation of total carbonate in soils, the method officially adopted when accurate results are desired is the modification of Reiset's



method described by Amos<sup>1</sup>. A known mass of the soil is treated with excess of fairly concentrated hydrochloric acid. The flask containing the soil and acid is slowly heated, and the expulsion of the last traces of carbon dioxide is ensured by subsequent boiling for about twenty minutes. The carbon dioxide evolved is absorbed by caustic soda solution contained in a Reiset tower, and its amount estimated by double titration, using first phenolphthalein and then methyl orange as indicator.

The value of this method is based on the reliability with which it can be used in determining the total carbonate content of soils which are on the border line of acidity. It enables the soil chemist in most cases to give trustworthy advice on the question as to whether a soil is in need of lime or not. The method, however, does not differentiate between easily decomposed carbonates and those which are more stable. Should a soil, low in total carbonate, contain an appreciable amount of dolomite or some chemically similar mineral, it seems possible that the figure obtained in the carbonate estimation might be misleading, for such a soil, appearing from chemical analysis not to be in immediate need of lime, might yet respond markedly to a dressing of that substance.

The inquiry naturally leads to several distinct lines of investigation, amongst which the following may be enumerated.

#### A. *Mineralogical.*

1. The treatment and examination of soils under the petrographical microscope in order to identify any carbonate minerals that might be present.

#### B. *Biological.*

2. The examination of the effect of using dolomite or similar mineral in place of calcium carbonate as a base for neutralising the acids liberated during nitrification in culture solutions.

#### C. *Chemical.*

3. The elaboration and standardisation of some simple chemical method of detecting the presence of dolomite etc. in a soil, and of estimating the percentage amount.

4. The application of the standardised method to an examination of soils derived from several types of rock, with the object of establishing the presence or absence of dolomitoid carbonates in these soils.

<sup>1</sup> Arthur Amos, "A Method for the Determination of Carbonates in Soils," *Journ. Agric. Sci.* 1, 1905, p. 322.

5. The examination of the top-soil and the corresponding sub-soil of several soil-types known to contain dolomitoid carbonate in order to detect any selective action due to the effect of continued crop growing on the various soil carbonates.

The following is an account of the results so far obtained by the writer, working along these lines. The subject has by no means been thoroughly explored, for the investigation had to be curtailed owing to certain unavoidable circumstances, so that many of the questions raised have not received the attention they perhaps deserve.

#### A. MINERALOGICAL INVESTIGATION.

A description of the methods used and the results obtained in this part of the work will not here be given in detail, since they will be published in the form of a separate geological paper<sup>1</sup>.

A sample of marine silt (Soil No. II) taken from the foreshore of the coast of the Wash near Friskney was examined microscopically, and in it the presence of dolomite was definitely established. Other carbonates identified were calcite and aragonite (in the form of tiny shell fragments, small pieces of broken oolitic grains, and occasional tests of certain foraminifera). Several grains, which might possibly be composed of ankerite or some similar ferriferous carbonate, were also noted.

An interesting possibility indicated by the appearance, size and crystalline shape of the dolomite crystals, is that the mineral may be of secondary and comparatively recent origin in the silt. It may possibly have been formed by deposition from sea-water during the sedimentation of the silt, or even during those subsequent periods of alternate wetting and drying caused by the flow and ebb of the tides.

#### B. BIOLOGICAL INVESTIGATION.

An attempt was made to carry out experiments similar to those performed by Warington in his classical researches on nitrification in soils<sup>2</sup>. Besides calcium carbonate, various other carbonates were used as bases to neutralise the acids liberated during the activities of nitrification bacteria growing in culture solutions.

It is not intended here to give a full account of the experiments conducted by the writer, or to attempt any final statement of results, for

<sup>1</sup> F. Hardy, "The Mineral Composition of the Modern Fenland Silt with special reference to Carbonate Minerals," which will appear in *Geol. Mag.*

<sup>2</sup> Warington, *Journ. Chem. Soc.* 1878, 1879, 1884, 1891.

the reason that only a very limited number of trials could be performed in the time at the writer's disposal.

The following provisional statements may however be made with regard to the general conclusions indicated by the experiments so far completed.

1. In all cases, pure calcium carbonate allowed nitrification to proceed normally in both its stages (*i.e.* ammonia to nitrite, and nitrite to nitrate).

2. Magnesium carbonate appeared to retard the second stage of the process (*i.e.* nitrite to nitrate), although apparently it did not inhibit it.

3. Pure dolomite appeared to retard appreciably the whole process of nitrification, and not to exhibit any preferential retarding effect on one stage more than the other.

4. Ferriferous dolomite (of ankerite type) seemed to retard nitrification very markedly.

It was thought possible that the ferrous iron in such samples of dolomite might be the inhibiting factor; hence experiments to test this possibility were initiated. The results of these experiments indicated that:

5. Soluble ferrous compounds (*e.g.*  $\text{FeSO}_4$ ), in amount equivalent to that present in the ferriferous dolomite previously used, did not prevent nitrification in the presence of pure calcium carbonate.

6. Insoluble ferrous compounds (*e.g.* siderite,  $\text{FeCO}_3$ ) also did not prevent nitrification in the presence of pure calcium carbonate.

It therefore appears that dolomite (especially ferriferous dolomite), possibly because of its comparative stability towards acids, retards the process of nitrification at least in so far as culture solution experiments are concerned. It seems reasonable to suppose that it may also do so in the soil.

#### C. CHEMICAL INVESTIGATION.

##### I. *A method of estimating approximately the amount of dolomitoid carbonate in a soil.*

An application of the known differences in the effect of weak acids on calcitoid and dolomitoid carbonate minerals first suggests itself as the basis of a method for their separate estimation in any material which might contain them. It forms the underlying principle of the method ultimately adopted by the writer.

The difference in the behaviour of calcite and dolomite towards

## E. HARDY

acetic acid has been applied by Linck<sup>1</sup> to the examination of certain rocks suspected of containing the two. Linck added a solution of ammonium phosphate to the dilute acetic acid used in his experiments. This acts on dolomite to form a protective coating of magnesium ammonium phosphate over the crystals, and thereby lessens the re-activity of the dolomite towards the acid.

Lemberg, and later Hinden<sup>1</sup>, describe a method for estimating calcite and dolomite in rocks, based on the behaviour of these minerals towards ferric chloride or copper sulphate. This latter method, however, does not seem to lend itself to the estimation of the minerals when they occur in small amount in a substance.

The bearing of the facts outlined above on the estimation of mineral carbonates in soils may now be considered.

The simplest way in which a weak acid may be employed in the determinations of the percentage amounts of the two kinds of carbonates in soils is to estimate first the total carbonate in a representative ground sample of the air-dried soil, and then to treat another portion of the sample with the weak acid so as to remove the carbonates which are the most easily decomposed. The liquid may then be filtered off, the soil washed and dried, and the percentage of residual carbonate estimated.

The above was the procedure followed in the experiments described below. The use of ammonium phosphate, as suggested by Linck, was omitted, since it might have led to unforeseen complications, and its action on dolomite might possibly have interfered to some extent with the decomposition of the dolomitoid carbonates in the second part of the operation in which hydrochloric acid is used. A series of comparative tests, however, might profitably be performed in order to decide these points.

There are several obvious objections to the above method of estimating the different soil carbonates. Of these there is one which may appear to be very serious, for it lies in the fact that in soil analysis we are dealing with mixtures of particles of different sizes and present in varying proportions. Amongst the particles of a soil there may be particles of mineral carbonates. In one soil-type, the mineral carbonate grains might perhaps be present chiefly in the coarser fractions; in another, they might occur mainly in the finer fractions. Thus in par-

<sup>1</sup> For a summary of the chemical tests for calcite and dolomite in rocks, and for references to literature on the subject, the reader is referred to A. Johannsen, *Manual of Petrographic Methods*, McGraw Hill Book Company, New York, 1918.

ticular the amount of dolomitoid carbonate which might possibly be decomposed by the weak acid during the time this is in contact with the soil, would largely depend on the fineness of subdivision of the dolomite in that soil. Another error which might arise is due to the fact that dolomite reacts with dissolved carbon dioxide (carbonic acid) to form soluble bicarbonates; and another to the possibility of the weak acid employed in the first stage of the process attacking certain soil components other than mineral carbonates.

Hence any method of the nature of the one outlined above, if adopted, must be understood to be quite arbitrary, for it possesses limitations similar to those of certain other already established methods used in soil analysis. It might be expected, however, to possess most value when employed in comparative tests.

The results obtained in the experiments described below will show how far these facts affect the investigation of certain problems connected with the carbonate content of soils.

The choice of the most suitable weak acid for use in the estimation of the more resistant soil-carbonates is the next question for consideration. A solution of citric acid used after the manner employed by Dyer in his researches on the availability of phosphates and potash in soils, at once suggests itself to the soil chemist. It was not used by the writer, however, and its behaviour, as well as that of certain other weak acids in experiments such as those to be described, still remains to be examined. In the following experiments  $N/3$  acetic acid was selected for reasons to be explained later.

*(a) The behaviour of  $N/3$  acetic acid towards calcite.*

A specimen of Iceland spar was reduced to powder and passed through a sieve whose meshes were 0.2 mm. in diameter. The powder after sieving was then subjected to a 75 seconds sedimentation process in pure water from the 7.5 cm. mark of a beaker, after the manner of a mechanical soil analysis. In this way a sample of "calcite sand" was obtained. The remaining fraction (the size of whose particles was below 0.04 mm.) was also collected and labelled "calcite clay."

0.5 gm. each of "calcite sand" and of "calcite clay" were introduced into separate flasks, and 100 c.c. of  $N/3$  acetic acid were added to each. The flasks were then shaken with a rotary motion, so that the contents were kept in a constant state of agitation. This necessitated each vessel receiving a few turns once every half minute. The average temperature of the laboratory throughout this and subsequent experi-

ments was about 8° C. The number of minutes which elapsed between the commencement of the shaking and the point at which all the calcite clay had disappeared was recorded, as was also the time required for the complete solution of the calcite sand.

It was found from a series of such experiments that the 0.5 gm. of calcite clay completely disappeared in six minutes. The 0.5 gm. of calcite sand however required 45 minutes of the treatment before it was entirely decomposed.

Now it was assumed that no soil likely to be examined in subsequent experiments would contain more than 5 per cent. of its mass of calcite sand grains. In other words, 10 or 12 gms. of the soil (a convenient mass with which to work) would not be expected to contain more than about 0.5 gm. of calcite sand.

Hence the following procedure for estimating the calcitoid and dolomitoid carbonates that might occur in a given soil should give (as far as the exactitude of the method allows), fairly trustworthy results.

(b) *Details of the method finally adopted for the estimation of the calcitoid and dolomitoid carbonates in certain soils.*

A representative sample of about 30 gms. of the air-dried soil is completely ground in a mortar so that it will pass through a dry sieve of mesh 0.2 mm. Two portions, each of mass about 12 gms. of the sieved soil, are taken. One of these portions is introduced into a flask along with 100 c.c. of CO<sub>2</sub>-free water, and the other is placed in a second flask with 100 c.c. of N/3 acetic acid. The two flasks are then shaken for 45 minutes in the manner previously described. After this time the contents of each flask are poured on to dry filters. The residues on the filters are washed with CO<sub>2</sub>-free water, and then dried together in an air oven at a temperature of about 30° C. When dry, 10 gms.<sup>1</sup> of each are weighed out and the percentage of carbonate in each is determined by the Amos method.

(i) The figure obtained with the water-treated soil is taken as the total carbonate percentage of the soil.

(ii) The percentage of carbonate (reckoned as CaCO<sub>3</sub>) in the acid-treated soil is taken as representing the dolomitoid carbonate.

(iii) The difference between the two values in (i) and (ii) is taken as representing the calcitoid carbonate.

<sup>1</sup> Or 5 gms. if the sample is high in carbonate content.

## NOTES.

(1) The figure obtained in (ii) does not of course accurately represent the percentage of dolomitoid carbonate in the original soil, but in the calcite-free soil.

(2) Similarly, the figure obtained in (iii) is not accurately the calcitoid carbonate percentage.

To obtain figures representing the exact percentages of the two sorts of carbonates in the soil, certain other experiments and calculations would have to be made (see below). In comparative tests however, and in experiments with soils whose total carbonate content is low, the figures obtained in (ii) and (iii) may be taken as representing approximately the dolomitoid and the calcitoid carbonate content. It is especially with soils in which the total amount of carbonate is small that the estimation of calcitoid and dolomitoid carbonates may be expected to prove of most value to the agriculturist, so that the error introduced in their case by an omission of the correcting calculation may not, after all, lead to serious misinterpretation.

(3) The tedium involved in shaking for a period of 45 minutes the flasks containing soil and liquid may constitute a possible objection to the method. It may be, however, that further trials will show that trustworthy results can be obtained if the time of shaking is made shorter. Unfortunately in the time at his disposal, the writer was only able to carry out a limited number of experiments on this point, and deemed it advisable at this stage to decide on a somewhat prolonged period of shaking in order to secure a margin of safety. It is clear, however, that any possible tedium is minimised when several determinations are being carried out simultaneously, for as many as seven soils, involving the use of fourteen flasks, may conveniently be treated in the manner described.

(4) The plan of using a water-treated sample of the soil in the estimation of the total carbonate reduces the experimental error somewhat, in that it corrects for the removal of water-soluble substances during the treatment, and also ensures that the paired samples used in the carbonate estimations are equally dry.

II. *Application of the method to the examination of certain "soils" artificially prepared by the addition of calcite and dolomite to a suitable matrix.*

In the specimens *A* to *E* described below, pure carbonate-free quartz sand (the size of whose particles lay between 0.4 and 0.02 mm.) was used as matrix.

*Specimen A.* Calcite sand, similar to that used in the last experiment, was added to the matrix in such amount that the mixture contained 5 per cent. of the carbonate.

*Specimen B.* Similarly, this specimen was made up so that it contained 4.4 per cent. of calcite sand and 2.2 per cent. of dolomite sand.

*Specimen C.* This contained 2 per cent. of dolomite sand and 1 per cent. of dolomite clay.

*Specimen D.* In this there were present calcite sand (4.54 per cent.), calcite clay (0.74 per cent.), dolomite sand (1.68 per cent.), and dolomite clay (0.25 per cent.).

*Specimen E.* The carbonate content of this consisted of calcite sand (0.44 per cent.), calcite clay (1 per cent.), dolomite sand (0.54 per cent.) and dolomite clay (1.28 per cent.).

NOTE.

The dolomite used in the above mixtures was a specimen which contained rather a high percentage of ferrous carbonate, so that it approximated to ankerite in composition. On analysis, it gave the following results:  $\text{CaCO}_3$ , 53.75 per cent.;  $\text{MgCO}_3$ , 4.97 per cent.;  $\text{FeCO}_3$ , 39.37 per cent., and silica etc. (by difference) 1.91 per cent.

The total percentage of carbonate (expressed as  $\text{CaCO}_3$ ) and the percentage of dolomitoid carbonate (also expressed as  $\text{CaCO}_3$ ) were determined exactly as described in the above account of the method.

In order to serve as a check on the results obtained, and to indicate the magnitude of the error involved in recording the results of the estimation as outlined in the method, the total amount of calcium carbonate in the filtrate from the acid-extracted "soil" was in each case estimated by Shrewsbury's method<sup>1</sup>.

Shrewsbury suggested a method for determining the carbonate content of soils, in which the calcium carbonate is first extracted by  $N/4$  acetic acid, in a manner similar to that independently adopted by

<sup>1</sup> H. S. Shrewsbury, "A Method of estimating Calcium carbonate in Soils," *Analyst*, xxxvii, 1912, p. 128.



the present writer. A known volume of the extract is then evaporated to dryness and ignited. The calcium oxide left is taken up with 10 c.c. *N*/10 acetic acid, and the excess of this acid finally estimated volumetrically, using *N*/10 sodium hydroxide with phenolphthalein as indicator.

Shrewsbury's method, of course, would not be expected to give completely accurate results with soils which contain an appreciable amount of dolomitoid carbonate.

Table I. *Results of Analyses of Artificial Soils.*

Artificial "Soil"	Percentage calcite	Percentage dolomite	Percentage dolomite (as $\text{CaCO}_3$ )	Total percentage $\text{CaCO}_3$ (sum of 1 and 3)	Experimental Results			
					Total percentage $\text{CaCO}_3$ found	Percentage dolomitoid carbonate (as $\text{CaCO}_3$ ) found	Percentage calcitoid carbonate (as $\text{CaCO}_3$ ) by difference	Percentage calcitoid car- bonate found (Shrewsbury's method)
Specimen A	5.00 (sand)	—	—	5.00	4.94	0.06	4.88	4.84
" B	4.40 (sand)	2.20 (sand)	2.06	6.46	6.40	2.25	4.15	4.50
" C	—	2.00 (sand) 1.00 (clay)	2.79	2.79	2.75	2.21	0.54	0.60
" D	4.54 (sand) 0.74 (clay)	1.68 (sand) 0.25 (clay)	1.81	7.09	6.90	1.66	5.24	5.51
" E	0.44 (sand) 1.00 (clay)	0.54 (sand) 1.28 (clay)	1.70	3.14	2.99	1.03	1.96	1.85

These results show that the method may be relied upon to give a fairly accurate indication of the presence of calcitoid and dolomitoid carbonates in any soils which may contain them, and also of the approximate percentage amount of each kind of carbonate. Further, it appears from the results that the figures obtained become more reliable as the total carbonate content of a soil approaches a minimum.

III. *An examination of certain soils of various geological origins, with a view to establishing the presence or absence of dolomitoid carbonate in them.*

The series of soil-types described and tabulated below were analysed according to the method previously outlined.

*Notes and Deductions (Table II).*

(1) The first series of soil samples (I, II, III and VI) are silt soils of the Wash area, which have not yet been reclaimed for agriculture. Their carbonate content has not therefore been exposed to the wastage action of certain processes which go on in cultivated soils. It appears

from the analytical figures that the calcitoid carbonate content of these soils is approximately twice that of the dolomitoid carbonate content (column 6).

Table II. *Results of Analyses of certain Soil-Types.*

sample	Description of Soil	Approximate physical composition (sand : silt : clay)	Percentage total carbonate (as CaCO <sub>3</sub> )	Percentage dolomitoid carbonate (as CaCO <sub>3</sub> )	Percentage calcitoid carbonate (as CaCO <sub>3</sub> ) by difference	Percentage calcitoid carbonate (by Shrewsbury's method)	Ratio dolomitoid carbonate carbonate
Soil	(a) <i>Marine silts.</i>	1	2	3	4	5	6
I	Bare foreshore silt, Wrangle, N.W. coast of Wash	41 : 32	10.90	3.25	7.65	7.95	2.35
II	Bare foreshore silt, Friskney, N.W. coast of Wash, 4 miles to S.W. of last	83 : 12	8.98	3.23	5.75	5.92	1.78
III	Green marsh silt, Wrangle	44 : 38	2.56	0.65	1.91	1.85	2.44
VI	Green marsh silt, Butterwick, N.W. coast of Wash, 4 miles to S.W. of last	30 : 46	9.86	4.35	5.51	—	1.26
XI	Bare foreshore silt, Meathop Marsh, Morecambe Bay, Westmorland	87 : 2	7.78	1.95	5.83	5.88	2.98
XIII	Poor natural pasture, Meathop Marsh, 1 mile from foreshore	80 : 9	1.16	0.56	0.60	—	1.06
	(b) <i>Other types.</i>						
XVI	Arable soil, derived in the main from Gault, Wretton, Norfolk	60 : 23	0.39	0.11	0.28	—	2.54
XVII	Pasture soil, derived from Chalky boulder clay, Halstead, Essex	43 : 41	3.72	0.39	3.33	—	8.54
XVIII	Arable soil, derived from Chalky boulder clay, Raveningham, S. Norfolk	52 : 33	5.24	0.01	5.23	5.20	523.00
XIX	Poor arable soil, derived from Pliocene crag, Ranworth, E. Norfolk	66 : 28	1.32	0.01	1.31	—	131.00
XX	Arable soil, derived from Chalky boulder clay, Great Melton, Essex	71 : 21	0.39	0.00	0.39	—	∞
XI	Arable soil, derived from Chalk, Sedgeford, Norfolk	—	9.81	0.00	9.81	—	∞

(2) Samples XI and XIII were collected by the writer from the alluvial flat which borders the estuary of the River Kent where it flows into Morecambe Bay. The flat was until recent times flooded by sea-water at the higher tides. It is evident that the Meathop alluvium was

deposited under conditions similar to those which obtained during the laying down of the Wash silts. Although the Meathop silt was not examined mineralogically, the resemblance between its carbonate content and that of certain of the Fenland silts as revealed by the chemical analyses, affords a reason for concluding that the Meathop silt contains dolomitoid carbonate minerals. This conclusion is strengthened by a further comparison of the analytical results given by other samples of silts procured from these two widely distant regions (see Table III).

(3) It appears, therefore, that marine sedimentary material (or soils derived from such material) formed under certain definite conditions, as exemplified by those conditions under which the Wash and the Meathop silts were deposited, may be expected to contain dolomitoid carbonate as part of the total carbonate content. Further investigations of marine alluvium and alluvial soils may establish this generalisation.

(4) Should it become recognised that marine alluvial soils on the whole are likely to contain the two sorts of carbonate specified, the conclusion may lead to the greater exercise of caution when deductions from the results of the analyses of such soils are being attempted by the soil chemist.

(5) The analysis of Soil XVI, if reliable, is in perfect agreement with the conclusions drawn by geologists as to the conditions under which certain facies of the English Gault beds were deposited.

(6) The sediments from which Soils XVII to XXI were derived seem to contain practically no dolomitoid carbonate. This fact again is in accord with the lithological characters and the geological history of the sediments in question.

IV. *An examination of certain soils containing dolomitoid carbonate, with a view to finding the effect of continued crop growing on the carbonate content of those soils.*

The soils examined in this part of the investigation were marine silt soils from the Wash area and from Meathop Marsh. They include soils which have been under cultivation for a very long time, and also soils which have been reclaimed in comparatively recent years. Hence a study of the analytical figures obtained by applying the method heretofore used for the detection and approximate estimation of the two kinds of soil-carbonates, should yield interesting and possibly important results.

Table III. *Results of Analyses of certain Cultivated Soils which contain Dolomitic Carbonate.*

Sample	Description of soil	Approximate Physical composition (sand : silt + clay)	Percentage total carbonate (as CaCO <sub>3</sub> )	Percentage dolomitic carbonate (as CaCO <sub>3</sub> )	Percentage calcitoid carbonate (as CaCO <sub>3</sub> )	Ratio dolomitic carbonate calcitoid carbonate	Difference between ratios for top- and sub-soil	
		1	2	3	4	5	6	
(a) <i>Marine silt soils of the N.W. coastal belt of the Wash.</i>								
Series of samples of soils arranged in order of increasing geological age. (Samples taken in a straight line proceeding north-eastwards from the shore near Butterwick to Frithville, distant 6½ miles.)								
Soil VI	(For comparison) Uncultivated green marsh, Butterwick	30 : 46	9.86	4.35	5.51	1.26	-0.14	
" VII	Arable land between the 1851 sea-bank and the Roman bank, Butterwick	43 : 36	8.95	4.23	4.72	1.12		
" VIII	Arable land between Butterwick and Benington, 1½ miles N.E. of latter	37 : 45	3.81	1.96	1.85	0.94	+0.33	
" IX	Arable land, 1 mile south of Silbsey, and 2½ miles due N.E. of the last area sampled	52 : 34	3.09	1.82	1.27	0.69		
" X	Arable land near Frithville, and 2½ miles due N.E. of the area of sample IX	49 : 37	3.60	2.09	3.51	1.68	+0.99	
" IV	Arable land between the 1851 sea-bank and the Roman bank, Wrangle	53 : 38	0.70	0.38	0.32	0.84		
" V	Arable land immediately to landward of the Roman bank, Friesion (5 miles S.W. of Wrangle)	49 : 37	2.40	0.74	1.66	2.23	+1.39	
"	Other examples.	55 : 35	0.50	0.33	0.17	0.51		
"		61 : 31	2.73	1.33	1.40	1.07	+0.56	
"		40 : 41	3.12	1.81	1.31	0.72	+0.61	
"		44 : 38	7.70	3.30	4.40	1.33		
"		66 : 22	3.22	2.11	1.11	0.52	+1.22	
"		65 : 23	6.20	2.26	3.94	1.74		
(b) <i>Marine silt soils of Meathop Marsh, Morecambe Bay, Westmorland.</i>								
Series of samples of soils arranged in order of increasing geological age. (Samples taken in a straight line proceeding north-westwards from the foreshore to the head of the alluvial flat, distant 1 mile.)								
" XI	(For comparison) Uncultivated foreshore, south part of the Marsh	87 : 20	7.78	1.95	5.83	2.98	-0.25	
" XII	Enclosed natural meadow, centre of Marsh	88 : 20	8.24	2.21	6.03	2.73		
" XIII	Poor pasture, north part of marsh, and ¾ mile from foreshore	80 : 11	5.29	0.72	0.48	0.66	+0.80	
" XIV	Arable land, extreme north of Marsh, to landward of low sea-bank	80 : 90	1.16	0.56	0.60	1.06		
"		84 : 70	5.77	1.85	3.92	2.12	+1.06	
"		74 : 15	0.44	0.36	0.08	0.22		
"		73 : 16	2.75	0.86	1.89	2.19	+1.97	
<i>Another example.</i>								
" XV	Arable land, Low Meathop, or Grange Marsh, in valley of R. Winster, 2 miles west of Meathop Marsh	84 : 60	4.25	1.34	2.91	2.17	+1.35	
"		87 : 30	8.00	1.77	6.23	3.52		

For purposes of comparison, the sub-soils corresponding to the samples of the top nine inches of the soils were submitted to the same analytical treatment as the top soils.

The figures obtained are set out in Table III.

*Notes and Deductions (Table III).*

(1) The first point to be noticed in the experimental results set out in Table III is that in the case of each soil sample in the list, there is a fair continuity between top-soil and sub-soil, as indicated by the mechanical analyses (column 1). One may perhaps justly infer that the layers of silt which now constitute the top-soil and the sub-soil in any one locality within either of the areas investigated, are approximately contemporaneous. This conclusion agrees with the observed mode of formation of silt lands such as are still being built up as "salt-ings" both along the coast of the Wash and on the shores of the estuaries which open into Morecambe Bay.

(2) If this continuity between the top-soil and the sub-soil of the two areas especially under consideration be granted, it becomes evident from the figures in the table that cultivation has caused, in the case of most of the soils analysed, a marked decrease in the total carbonate content (column 2).

(3) This decrease in total carbonate due to crop growing has in general been greatest in those soils which have been longest under cultivation. This is what would be expected.

(4) When we come next to consider the "relative wastage" of the calcitoid and the dolomitoid carbonate content of each soil, it will be seen that the more easily decomposed carbonate has been removed at a quicker rate than the less easily decomposed carbonate.

The ratio of calcitoid to dolomitoid carbonate (column 5) in the case of any one top-soil may be taken to represent the relative wastage of the first to the second. Consider, for example, Soil VII. Here the ratio for the sub-soil is 1.27, which implies that the sub-soil contains about one and a quarter times as much calcitoid carbonate as dolomitoid carbonate. In the top-soil of Sample VII, however, the ratio has been reduced to 0.94, so that cultivation has apparently caused the removal of calcitoid carbonate at a rate greater than that at which dolomitoid carbonate has been removed.

(It is to be understood that the actual value which the ratio possesses in any one case is not to be taken as having any strictly mathematical significance, for it is arrived at in the first place by a comparison of

two figures which have been obtained by an approximate experiment only, and secondly, it is based on the assumption that the top-soil and sub-soil originally contained similar amounts of the two sorts of carbonates.)

(5) With the reservations made in the last paragraph it becomes clear by an inspection of the figures in the last two columns that the rate of calcitoid carbonate wastage relative to that of dolomitoid carbonate is in general greatest in those soils which have been for the longest time under cultivation. This again is in accordance with expectation, taking into account the chemical differences between the calcitoid and dolomitoid carbonates.

(6) The figures obtained for the total carbonate content of the sub-soils in the two series of increasing geological age and length of agricultural treatment indicate a general falling off in the amount of carbonate. This fact might be interpreted to mean that the carbonate content of the sub-soils in the case of each sample, like that of the top-soils, is also being lessened by the climatic and biological agencies at work. This conclusion cannot be held to be incontrovertible, however, since the variations could also be explained by assuming that, for some reason or other, the silt deposited at the commencement of the formation of the alluvial areas was poorer in carbonates than that which accumulated at a later date. The question could be settled only by the analyses of samples taken at successive depths below  $1\frac{1}{2}$  feet (which is the lower limit of the sub-soil as understood by the soil chemist).

(7) An important bearing of the analytical figures contained in Table III is in connection with the carbonate content of soils on the border-line of deficiency of carbonate. Reference to the figures obtained in the analyses of Samples IX, X, XII, XIII and XIV, indicates that these soils are likely to benefit by dressings of lime, for their percentages of calcitoid carbonate are of the order 0.5 per cent. or under, so that the soils appear to be in danger of becoming "sour." This question is dealt with in more detail in the next section.

*V. An examination of certain soils which appear from the results of the last experiment to be on the verge of deficiency of carbonate.*

Determinations were made of the "lime requirement" of certain of the soils of the series last examined in which the percentage of calcitoid carbonate is below 0.75. The Hutchinson-McLennan method<sup>1</sup> was employed.

<sup>1</sup> H. B. Hutchinson and K. McLennan, "Studies on the Lime Requirements of Certain Soils," *Journ. Agric. Sci.* vii, 1915, p. 75.

Table IV. *The Results of the Lime-requirement Determinations.*

Sample	Percentage loss from carbonate	Percentage dolomite carbonate	Percentage calcite carbonate	Ratio calcite dolomite	Acidity to lit- mus solution	Lime requirement	
						Vol. N/10 HCl*	Lbs. per acre required to correct deficiency†
Soil XII	1.20	0.72	0.48	0.66	Neutral	+0.20	—
„ XIII	1.16	0.56	0.60	1.06	Neutral	0.00	—
„ IX	0.70	0.38	0.32	0.84	Distinctly acid	-0.15	19 lbs.
„ X	0.50	0.33	0.17	0.51	Distinctly acid	-0.55	69 lbs.
„ XIV	0.44	0.36	0.08	0.22	Very distinctly acid	-2.05	2562 lbs.

\* *The Hutchinson-McLennan method.*

20 gms. of soil were shaken with 200 c.c. dilute calcium bicarbonate solution for 30 minutes (only); 100 c.c. were then filtered off and titrated with N/10 HCl, using methyl orange as indicator. The differences between the volumes of N/10 HCl needed to neutralise 100 c.c. of the original bicarbonate solution, and 100 c.c. of the filtrates after shaking with soil, are recorded in this column.

† The average weight of 1 acre of silt soil of depth 9" is here taken as 2½ million pounds.

*The vegetation of the soil areas to which the samples correspond.*

*Soil XII.* Enclosed natural meadow land, centre of Meathop Marsh, Westmorland.

*Dominants.* Fiorin (*Agrostis stolonifera* and *alba*), Crested Dogtail, White Clover.

*Sub-dominants.* Hawkbit, Plantain (*P. maritima* and *coronopus*), Yorkshire Fog, Couch (*Agropyrum junceum*).

*Soil XIII.* Poor pasture, north part of Meathop Marsh. The flora is very much like the last, but the individual plants are not so well grown since the land has been continuously grazed, and has never received dressings of manure as has the meadow.

*Soil IX.* Arable land, Sibsey, Fenland.

*Soil X.* Arable land, Frithville, Fenland.

There are no available records of the agricultural and weed flora of these districts.

*Soil XIV.* Arable land, north of Meathop Marsh. Oats and mangels give good crops on this soil, but White Clover grows poorly and needs dressings of basic slag to give reasonable yields. The land has received no slag since 1915. The weed flora includes Yorkshire Fog, Sorrel, Spurry and other plants which are generally taken to indicate an "acid" condition in soils.

*Notes and Deductions (Table IV).*

(1) It appears from the above results that when marine silt soils containing dolomitoid minerals are examined for deficiency in calcium carbonate, those soils which contain anything less than about 1.0 per cent. of total carbonate (expressed as  $\text{CaCO}_3$ ) may be expected to indicate a need for liming.

(With most soils, this percentage (1.0 per cent.) of total carbonate would usually be considered as furnishing sufficient laboratory evidence of the absence of any immediate necessity for liming.)

(2) In the soils examined for lime requirement, those soils in which the ratio "calcitoid to dolomitoid carbonate" is lowest are the most "acid." This indicates that, although dolomitoid carbonates are gradually decomposed in soils, yet their rate of wastage is much less than that of the calcitoid minerals and does not apparently keep pace with the demand for an easily neutralisable base during the progress of certain essential soil reactions. Hence "acidity" might accumulate so that the soil comes to exhibit certain characteristics which are included under the general term "sourness."

In conclusion the author wishes to express his indebtedness to his colleague, E. J. Roberts, of the Cambridge School of Agriculture, for permission to use several unpublished analyses of silt soils of the Wash coastal area, and for samples of silt soils collected by him. Also to his colleague, L. F. Newman, M.A., for samples of soils obtained during his survey of the soils of Norfolk, and for much kindly criticism and help. Finally, the author wishes to acknowledge his gratitude to Professor Wood, C.B.E., M.A., F.R.S., for permission to perform the laboratory work connected with this investigation in the School of Agriculture, Cambridge.

## SUMMARY.

1. A sample of marine silt taken from the foreshore of the N.W. coastal belt of the Wash was found as the result of a mineralogical examination to contain dolomite in its mineral assemblage.

2. It was surmised from this discovery that should dolomite be a normal constituent of soils derived from marine silts, its presence might modify in them certain processes such as nitrification in which the neutralising effect of a quick acting base is an essential factor.

3. This supposition was strengthened by certain culture experiments on nitrification, and was to some extent established as a fact by a



detailed chemical investigation into the nature of the carbonate content of several soils of known geological and agricultural history.

4. A method for the approximate estimation of easily decomposed ("calcitoid") carbonate, and of more stable ("dolomitoid") carbonate in soils was elaborated and applied in the chemical investigation mentioned.

5. From the results of the chemical experiments it appears that

(i) Marine silt soils may be expected to contain both calcitoid and dolomitoid carbonate.

(ii) Soils not directly or indirectly derived from marine silts usually do not contain dolomitoid carbonate, although they might contain calcitoid carbonate.

(iii) Cultivation for crop production reduces both the calcitoid and the dolomitoid carbonate content of soils containing both kinds of carbonate, but the rate of wastage of the former is considerably greater than that of the latter.

(iv) "Sourness" in soils originally containing both kinds of carbonate, may occur when the total carbonate content is apparently well above the usual value taken by soil chemists as indicating deficiency of carbonate. This may be explained by the relatively greater stability of dolomitoid carbonate towards weak acids such as are supposed to accumulate in many cases of soil acidity, for such acids may only be quickly neutralised by the less stable calcitoid carbonate.

6. Marine silt soils and soils derived from them should receive special consideration from the soil chemist when problems relating to their carbonate content are under investigation.

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## STUDIES ON SOIL REACTION. I.

### A RÉSUMÉ.

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#### IMPORTANCE OF SOIL REACTION AS A FACTOR IN SOIL FERTILITY.

SOIL fertility is often spoken of as though it were an absolute property of the soil; in reality it is a particular relationship subsisting between *soil conditions* on the one hand and *crop growth* on the other. The conditions conducive to soil fertility are the resultant of two main groups of factors, the *intrinsic* properties of the soil which are dependent on its chemical, physical and biological nature, and those *extrinsic* properties impressed on the soil by topographical and climatic factors\*. These two groups cannot be sharply distinguished from each other, nor can any hard and fast line be drawn between the various factors comprised in them, because few are at present susceptible of exact measurement. In other words, that particular group of conditions that make up the fertility of a soil is an equilibrium brought about by the interaction of numerous factors some of which however can be varied by the agriculturist by manurial and cultivation operations.

Among those soil factors that are most readily controllable are many that cannot vary beyond comparatively narrow limits without becoming limiting or controlling factors in crop production. In particular the growth of soil organisms, as well as that of the plant itself, is very sensitive to the reaction of the medium, and it may happen that the presence or absence of a base will act as a limiting factor in crop production, and not merely through the direct effect of acidity or alkalinity on the plant itself or on the soil organisms, but on account also of the varying displacements produced on many of the factors that go to make up the complex chain of soil equilibria.

The question of soil reaction is therefore of great importance and one aspect of it, *viz.* soil acidity, or "sourness" to use the farmers'

\* For an interesting discussion of fertility from this point of view, see E. J. Russell, *Soil Conditions and Plant Growth* (Longmans), 3rd ed. 1917, especially chap. VII.

phrase, has attracted attention from very early times. The use of lime—as oxide, hydroxide or carbonate, the last named as chalk, limestone or marl—in correcting soil acidity, or adjusting soil reaction, is among the oldest of agricultural operations; while of late years the study of soil acidity has occupied an important place in agricultural research. It cannot be said, however, that the enormous amount of work done has either solved the practical problem or clarified our ideas as to what exactly soil acidity means; and matters are not simplified by the use of a multiplicity of such terms as “soil acidity,” “apparent acidity,” “real acidity,” “potential acidity,” “adsorption acidity,” “positive” and “negative” acidity, “lime requirements” of soils and of plants, “immediate” and “continuous” lime requirements, “active” and “latent” lime requirements, etc.

#### THEORIES OF SOIL ACIDITY.

Some soils are apparently so acid that when moist they will redden litmus paper almost immediately, but their aqueous extracts seldom redden litmus after boiling off the  $\text{CO}_2$ . The comparatively few cases reported in which the  $\text{CO}_2$ -free aqueous extracts were acid to litmus have invariably been the result of highly abnormal conditions\*, and the immediate cause of the reaction is obvious. Most acid soils, however, yield so little soluble acid on extraction with water alone that it cannot usually be detected by litmus paper after boiling off  $\text{CO}_2$ .

##### *A. Humic Acid Theory.*

Various theories have been put forward at different times to explain such acidity as this. Sprengel<sup>(69)</sup> in 1826 attributed the acidity to the accumulation of insoluble complex organic acids—the so-called humic acids—produced by the decomposition of plant residues left over from the crop. The dark alkaline solution obtained on treating an acid soil with ammonia was supposed to contain the soluble ammonium salts of these acids and the acids themselves could be precipitated on acidifying. Such acids were also supposed to occur in neutral and alkaline soils combined with calcium or magnesium and are then practically insoluble in alkalies, without a previous extraction with acid. This hypothesis long held the field. The compounds obtained from soils, however, were very indefinite and variable in composition and

\* For examples of such see Blair and Macy (12); Abbott, Connor and Smalley (1); Ruprecht (62), and Ames and Schollenberger (4). The numerals refer to references collected at the end of the paper.

always contained mineral impurities that could not be eliminated. Doubts therefore arose as to whether these so-called humus acids really were definite chemical compounds.

### *B. Selective Adsorption Theory.*

The first real advance was due to van Bemmelen<sup>(10,11)</sup> (1888) who considered that these bodies were not definite chemical compounds but absorption complexes, *i.e.* mixtures of a base and of various colloidal substances held together by some sort of surface attraction. These ideas were further developed into a general theory of soil acidity by Baumann and Gully about 1910<sup>(8,9)</sup>. The acid reaction of peat moss and of peat soils was attributed by these authors to the colloidal matter in the coverings of the hyaline sphagnum cells. The original sphagnum was about as "acid" as the peat, hence it is unnecessary to assume that the "acid" is a decomposition product. Moreover, if an acid is really present it is very insoluble as an aqueous extract of peat is practically neutral to litmus; and this is generally true of most "acid" soils. These views were based on the work of Linder and Picton<sup>(42)</sup> who showed that when  $As_2S_3$  is precipitated from colloidal solution by  $BaCl_2$  a small amount of barium is carried down with it and a corresponding amount of HCl is set free. If Ca-, Sr-, or K-chloride is used the same amount of HCl is left behind showing that equivalent amounts of the base are absorbed in each case. The Ba thus carried down is held very firmly and cannot be removed by washing although it is easily removed by other bases by digesting with an appropriate salt in solution. The work of Whitney and Ober<sup>(80)</sup> concerning the electrical condition of colloidal solutions gave some theoretical basis to Baumann and Gully's views, and further support was accorded by the fact that acidity is developed on shaking an acid or a neutral soil with neutral salt solutions. This phenomenon was first noticed by Thompson<sup>(73)</sup> and was explained by Way<sup>(78)</sup> as due to an interchange of bases between the neutral salt and a constituent of clay—a hydrated alumino-silicate of an alkali or alkaline earth. Later when the general theory of surface adsorption had been put forward and developed by Willard Gibbs, J. J. Thomson, Freundlich<sup>(23)</sup> and Wo. Ostwald<sup>(56)</sup> it was extended and applied in a somewhat modified form and under the name of selective adsorption to soil interchanges by Cameron<sup>(15,16)</sup> and later by E. G. Parker<sup>(57)</sup> and by Harris<sup>(29,30)</sup>. Parker found that when an acid soil is shaken with a solution of KCl, the potash was displaced by aluminium and other bases in nearly equivalent amounts, the Cl remaining unchanged. When NaOH was

also present with the KCl just as much potash was absorbed by the soil but no bases from the soil replaced it. Parker concluded that the base is adsorbed by the soil and a real acidity developed which then dissolves from the soil the bases found in the solution. This conclusion was supported by washing out the soluble bases from the soil with HCl, washing the soil free from HCl and treating with  $\text{KNO}_3$  solution: a considerable amount of free  $\text{HNO}_3$  was found in the solution besides the usual  $\text{Al}(\text{NO}_3)_3$  etc. Further support is given to this view by the more recent work of Blum<sup>(13)</sup> and Knight<sup>(38)</sup>.

### *C. Basic Exchange Theory.*

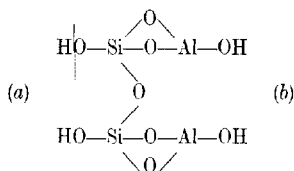
This adsorption view is not, however, universally held and many still hold that a real interchange of bases occurs not however with the insoluble organic acids of the soil but with the complex alumino-silicates of the clay fraction. This modern development of the classical work of Way<sup>(78)</sup> and that of Lemberg<sup>(40, 41)</sup> on the transformation of minerals by contact with salt solutions has much to support it. Sullivan<sup>(68)</sup> points out that  $\text{CaCl}_2$  and an artificial Na-alumino-silicate may interact to form two slightly soluble substances, Na- and Ca-alumino-silicates. In such a case the reaction does not proceed to the formation of one of these two substances exclusively, but an equilibrium is reached in which both are present; a definite relationship prevails between the concentrations of the reacting substances in the solution: the amounts of different bases absorbed will vary with the solubility of their respective alumino-silicates, while change in temperature, by shifting the relative solubilities, may lead to readjustments in the equilibrium. Such an absorption is in general an exponential function of the concentration as also are the solubilities of the alkaline earth sulphates in acids of varying concentration (Ostwald<sup>(55)</sup>) and the action of  $\text{K}_2\text{CO}_3$  on  $\text{BaSO}_4$  (Meyerhoffer<sup>(51, 52, 53)</sup>). This form of curve is not therefore necessarily a proof of adsorption\*. Such interchanges occur with many other silicates and alumino-silicates and sometimes Al is replaced by the base of a neutral salt: this accords with Veitch's<sup>(75)</sup> conclusion that an interchange of bases occurs between the neutral salt and the hydrated neutral silicates or alumino-silicates of the soil by which Al is brought into solution, the acidity developed being due to the hydrolysis of the Al salt. Daikuhara<sup>(21)</sup> and also Rice<sup>(60, 61)</sup> came to somewhat similar conclusions and this aspect has been further developed by the work of Spurway<sup>(70)</sup>

\* This point cannot be too strongly emphasised; see Walker and Appleyard<sup>(76)</sup> and especially Moore<sup>(54)</sup>.

on the "hydrolytic ratio" of soils, and that of Hartwell and Pember(31) on the part played by the  $Al^+$ -ion in the toxicity of acid soils. This however is probably not the whole story and there is much evidence to show that both interchange of bases and true selective absorption may occur together in the same soil although possibly with different soil constituents. The work of Rice(60, 61) and of Knight(38) seems to support this conclusion and moreover the very complexity of the soil militates against any single factor being the sole cause of soil acidity.

#### D. Mineral Acid Theory.

A fourth theory proposed by O. Loew(46) attributes the acidity of certain Porto Rican clay soils deficient in humus to an aluminosilicic acid in the clay which he calls "argillic acid" and to which he gives the formula



By the neutralisation of the acid OH groups at (a) the acid would become neutral, while absorption of  $P_2O_5$  is attributed to the basic OH groups at (b). By prolonged treatment of neutral clay, *i.e.* salts of argillic acid, with large quantities of water charged with  $CO_2$  an acid clay may be produced. The reverse action should also take place, *i.e.* neutral salts should be decomposed by acid clay the base being absorbed and the acid set free; this was actually observed by Daikuhara(21). The work of Ashley(7), *cf.* Mellor(50) and of F. W. Clarke(19) on the constitution of pure clays supports at any rate the plausibility of the theory and Truog(74) also emphasises the importance of soil silicates in absorption phenomena. It has however never been shown that the phenomena cannot be explained equally well by selective absorption or by interchange of bases.

#### METHODS OF DETERMINING SOIL ACIDITY.

Whatever the cause or causes of "soil acidity" it is difficult to avoid the conclusion that a certain degree of real acidity is present which

should be capable of measurement. Many methods have been proposed at various times\*. Among them are:

1. Inversion of cane sugar<sup>(61)</sup>.
2. Saponification of ethyl acetate<sup>(20)</sup>.
3. Liberation of iodine from a mixture of
  - (a) Potassium iodide and potassium iodate<sup>(8, 27, 28)</sup>.
  - (b) Potassium iodide and potassium nitrate<sup>(45)</sup>.
  - (c) Potassium iodide and potassium nitrite<sup>(21)</sup>.
4. Basic exchange<sup>(60)</sup> with
  - (a) Neutral salts<sup>(21, 32, 33, 46)</sup>, and
  - (b) Salts of weak acids<sup>(35, 36, 46)</sup>.
5. Absorption of base from a solution of
  - (a) Basic hydroxide<sup>(1, 14, 47, 67, 75, 79)</sup>, and
  - (b) Dye<sup>(37)</sup>.

Table I. *Comparison of various methods for determining Lime Requirement.*

CaCO<sub>3</sub> expressed as percentage of air-dried soil.

A. *Hutchinson's (34) results.*

Method used	Chelsea	Millbrook		Oundle	Woburn		Craibstone	
	CaCO <sub>3</sub> %	CaCO <sub>3</sub> %	Relative value	CaCO <sub>3</sub> %	CaCO <sub>3</sub> %	Relative value	CaCO <sub>3</sub> %	Relative value
Jones ...	0.045	0.045	225.0	0.018	0.232	89.2	0.161	35.1
Hopkins ...	0.012	0.006	30.0	0.002	0.244	94.0	0.030	7.0
Lyon and Bizzell	—	—	—	—	0.226	87.0	0.436	101.4
Veitch ...	—	—	—	—	0.204	80.0	0.407	94.6
Hutchinson ...	Nil	0.620	100.0	Nil	0.260	100.0	0.430	100.0

B. *Stephenson's (71) results.*

Method used	Gray silt loam		Brown silt loam	
	CaCO <sub>3</sub> %	Relative value	CaCO <sub>3</sub> %	Relative value
Tacke	0.215	100.0	0.325	100.0
Hopkins	0.100	46.5	0.120	36.9
Jones	0.193	89.7	0.241	74.2
MacIntire	0.184	85.4	0.203	62.6
Veitch	0.232	108.1	0.316	97.4
Truog	0.610	283.7	0.797	245.2

\* A short description of some of these methods is given by Hutchinson and MacLennan (34), a more critical examination of these methods, together with a résumé of all previous work on soil acidity, is given by Ames and Schollenberger (5) and by Frear (22).

Fertiliser used	1 None		2 Acid phosphate		5 NaNO <sub>3</sub>					
	a	b	a	b	a	b				
Method	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required				
Hopkins	0.172	117.6	0.005	5.9	0.1320	97.8				
Veitch	0.100	68.4	Alk.	—	0.100	74.1				
MacIntire	0.177	121.3	0.112	132.3	0.192	142.6				
Vacuum	0.365	249.6	0.245	288.2	0.387	288.9				
Hutchinson	0.146	100.0	0.085	100.0	0.135	100.0				
Plot No.	11	24	26	29	18					
Fertiliser used	11 Acid phosphate + mineral potash + NaNO <sub>3</sub>		24 Acid phosphate + mineral potash + Am <sub>2</sub> SO <sub>4</sub>		26 Bone meal + mineral potash + NaNO <sub>3</sub>		29 Basic slag + mineral potash + NaNO <sub>3</sub>		18 Farmyard manure	
	a	b	a	b	a	b	a	b	a	b
Method	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required	Percentage CaCO <sub>3</sub> required
Hopkins	0.154	109.0	0.004	5.8	0.154	109.0	0.146	130.7	0.1275	113.8
Veitch	0.080	63.7	Alk.	—	0.100	88.9	0.100	88.9	0.060	53.3
MacIntire	0.192	134.7	0.125	181.8	0.185	164.4	0.145	218.9	0.180	160.0
Vacuum	0.355	251.3	0.295	429.1	0.367	326.7	0.202	305.6	0.330	293.3
Hutchinson	0.141	100.0	0.066	100.0	0.112	100.0	0.066	100.0	0.112	100.0

b = limed in 1903 and 1909 to extent of 5,700 lbs. CaCO<sub>3</sub> per acre; lime requirements determined in 1912.

a = unlimed plots.

a = unlimited plots. b = limed in 1903 and 1909 to extent of 5,700 lbs. CaCO<sub>3</sub> per acre; lime requirements determined in 1912.



6. Decomposition of
  - (a) Insoluble(66, 72, 79, 82), and
  - (b) Soluble carbonates(34, 48).
7. Growth of *Azotobacter* in mannite(17) and of *B. mycoides* and *B. subtilis* in bouillon(43).

All these various methods give different results: the degree of acidity measured by some may be anything up to ten or twenty times that obtained by others. Examples are given in Table I, pp. 24, 25.

The conditions under which the various methods for estimating soil acidity are carried out vary so much among themselves and differ so enormously from the natural conditions in the field that the equilibrium approximately attained prior to the experiment would certainly be shifted in one direction or another during the determination. Thus Sharp and Hoagland(64) and Christensen(17) showed that the extraction of a soil with neutral salt solution, *e.g.* KCl or Ca-acetate, may actually change the reaction of the soil solution from distinctly alkaline to strongly acid, while in practically all cases extracts prepared with neutral salt solutions showed a higher hydrogen-ion concentration than extracts prepared with water alone. Moreover if a soil is extracted with Na-acetate solution of varying concentrations it has been found that the amount of acetic acid liberated increases while the actual hydrogen-ion concentration in the resulting solution diminishes as the concentration of the acetate increases. If the extraction is carried out with Na-acetate and NaCl solutions of equivalent strength then more titratable acid is liberated in the former case, but a higher hydrogen-ion concentration is attained in the latter.

#### SOIL ACIDITY A PHASE OF THE BROADER QUESTION OF SOIL REACTION.

In view of the above considerations it is permissible to ask of what value the usual laboratory methods can be as means of measuring soil acidity. At the best they can give some empirical information to the effect that certain soils examined will or will not be benefited by liming. They give—and can give—no quantitative information as to the degree of acidity in its strict, scientific and only possible meaning, *i.e.* the hydrogen-ion concentration of the nutrient solution bathing the soil particles. And yet the hydrogen-ion concentration of the soil solution, which is in equilibrium with the reserve of acidity bound up with the soil mass, is one of the few soil factors susceptible of investigation by really accurate methods. Until comparatively recently soil acidity has

been studied as though it were a thing apart and utterly unrelated to the ordinary physico-chemical concepts of acidity, the reason being, apparently, that the practical agricultural problem is complex involving colloid and physical factors as well as considerations of acidity in the physico-chemical sense,—i.e. as a function of the hydrogen-ion concentration. Soil acidity, in this narrower sense, thus becomes a phase of the broader question of soil reaction in general. Soil acidity is generally regarded as a pathological condition of the soil which may, and should, be removed by liming. This is not universally the case however. In potato growing an acid condition of the soil is beneficial, and is in fact generally preferred because it is not only not injurious to the potato crop but is inimical to the organism causing soft scab, *Actinomyces scabies* (*chromogenus*)<sup>(24)</sup>—one of the worst of potato pests. This disease never appears on a soil of a certain degree of acidity<sup>(25)</sup> and this work has met with some response in agricultural practice (Lipman<sup>(44)</sup>)\*.

#### SOIL ACIDITY CORRELATED WITH PHYSICO-CHEMICAL CONCEPTIONS OF ACIDITY.

##### A. Ionisation and dissociation constants of acids.

##### *Acidity as a function of [H<sup>+</sup>].*

At the outset soil acidity should be correlated with physico-chemical conceptions of acidity: all phenomena depending on acidity, whether in the soil or in any other medium, homogeneous or heterogeneous, are determined by the concentration of the hydrogen-ions in the continuous liquid phase. "Acidity," "alkalinity" and "neutrality" have a definite and quantitative meaning and refer to the ratio of the hydrogen-ion and hydroxyl-ion concentrations in the solution. All acids dissociate, or ionise, in aqueous solution thus— $\text{HA} \rightleftharpoons \text{H}^+ + \text{A}'$ ; this ionisation is quantitative and balanced and for a given temperature

$$\frac{[\text{H}^+] \times [\text{A}']}{[\text{HA}]} = K_A, \text{ a constant}^\dagger.$$

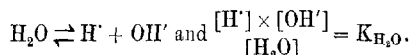
\* The work of Coville (*U.S. Dept. of Agric. Bul. 6, 1913*) on acid land agriculture is also of interest in this connection.

† The square brackets indicate concentration terms in gm.-ions or gm.-molecules per litre. This equation applies strictly only to *weak* acids. The "anomaly of strong electrolytes," however, need not concern us here as (1) the above equation does hold strictly for weak electrolytes, and (2) on account of "buffer effects" the soil acids can be regarded as weak electrolytes regardless of their actual chemical composition.

K is called the *ionisation constant* of the acid. Similarly for a base  $\text{BOH} \rightleftharpoons \text{B}' + \text{OH}'$ ; whence

$$\frac{[\text{B}'] \times [\text{OH}']}{[\text{BOH}]} = K_B, \text{ the ionisation constant of the base.}$$

The solvent—water—is regarded as being itself ionised to some small extent so that



The phenomena of hydrolysis are dependent on this ionisation of water which however is very small. In the case of water  $[\text{H}'] = [\text{OH}']$  and the neutral reaction is due to the equivalent concentrations of the hydrogen- and hydroxyl-ions. In any solution “acidity” is due to a preponderance of H-ions over OH-ions and the degree, or intensity, of acidity can be quantitatively expressed by the ratio  $[\text{H}']/[\text{OH}']$ . Conversely the presence of OH-ions in greater concentration than the H-ions renders a solution alkaline and the degree or intensity of alkalinity can be quantitatively expressed by the ratio  $[\text{OH}']/[\text{H}']$ . This is the only interpretation that ought to be placed on the expression “acidity” or “alkalinity” or more generally the “reaction” of a medium. In pure water at 18° C. or in absolutely neutral aqueous salt solution  $[\text{H}'] = [\text{OH}'] = 1 \times 10^{-7.07}$  g-ions per litre and  $[\text{H}'] \times [\text{OH}'] = 1 \times 10^{-14.14}$ . At constant temperature, then, the ionic product  $[\text{H}'] \times [\text{OH}']$  of any aqueous solution is a constant; any solute, such as an acid, that increases  $[\text{H}']$  will decrease  $[\text{OH}']$  and any solute, such as a free base, that increases  $[\text{OH}']$  will diminish  $[\text{H}']$ . But it is more convenient and much simpler, instead of expressing acidity in terms of  $[\text{H}']$  and alkalinity in terms of  $[\text{OH}']$ , to express both in terms of  $[\text{H}']$  alone. Neutrality would then correspond to a hydrogen-ion concentration of  $1 \times 10^{-7.07}$  gms. per litre, acidity to a  $[\text{H}']$  higher than and alkalinity to a  $[\text{H}']$  less than  $1 \times 10^{-7.07}$ .

#### B. *Acidity as a function of* $-\log [\text{H}']$ .

A further simplification is generally effected by regarding intensity of acidity as a function, not of the hydrogen-ion concentration, but of the reciprocal of the logarithm of the hydrogen-ion concentration. It is not easy at first sight to compare such a series of hydrogen-ion concentrations as

$$1 \times 10^{-10}; \quad 4.0 \times 10^{-10}; \quad 1.6 \times 10^{-11}; \quad 6.3 \times 10^{-12};$$

by a simple conversion, however, we get the following series

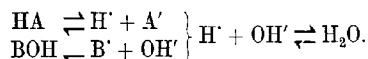
$$\begin{aligned} 1.0 \times 10^{-10} &= 10^{-10}; & 1.6 \times 10^{-11} &= 10^{-10.8}; \\ 4.0 \times 10^{-10} &= 10^{-9.4}; & 6.3 \times 10^{-12} &= 10^{-11.2}; \end{aligned}$$

and the order of the acidities is at once apparent\*. It thus becomes simpler to omit the 10, which is common to all, and to express the  $[H']$  by means of the *minus* logarithms which are merely the reciprocals of the logarithms of the hydrogen-ion concentrations and are generally written  $-\log [H']$  or  $P_H$ †.

A further advantage of this mode of expression is found in expressing graphically on squared paper large variations in  $[H']$ . It is impossible for example to compare graphically on the same sheet of paper  $[H'] = 10^{-9}$  and  $[H'] = 10^{-14}$  gms. per litre or any really large variation between these limits; but it is perfectly easy to compare the minus logarithms of these values, viz. 9 and 14 and any variations of any magnitude between these values.

### C. *Distinction between degree of acidity and titratable acidity.*

The degree of acidity as expressed by the hydrogen-ion concentration is of course not the same as that measured by titration. In the latter case hydrogen-ions are continuously removed by the alkali used in the titration, thus



The equilibrium is continually being shifted and as continually re-adjusted by the dissociation of more acid until all the acid has been

\* When the  $[H']$  is known the corresponding  $[OH']$  can be easily found, when necessary, by dividing the former into the ionic product. Thus when  $[H'] = 10^{-10.8}$  the corresponding  $[OH']$  will equal  $10^{-14.14}/10^{-10.8} = 10^{-3.34}$  g.-ions per litre.

† This method of expressing  $[H']$  was introduced by Sørensen(65) who substituted the term  $P_H$  for the older and more logical term  $-\log [H']$ . There would appear to be little justification for the introduction of the term  $P_H$ , although it has been adopted and widely used by many workers in agricultural science and in physiology. In pure physical chemistry the older term is in general use; its meaning is self-evident and perfectly unambiguous to any chemist while the term  $P_H$  requires explanation and may not always be quite free from ambiguity; thus in chemical kinetics  $p$  is the symbol generally employed to denote gaseous pressure and  $P_H$  would be the partial pressure due to hydrogen in a gaseous mixture. In the kinetic treatment of the theory of solutions gaseous pressure is replaced by osmotic pressure, the symbol for which is  $P$ . In this case  $P_H$  would denote the osmotic pressure due to hydrogen-ions which although a function of  $[H']$  can hardly be regarded as identical with the minus logarithm of the same. In this paper the older term  $-\log [H']$  will be used throughout.

dissociated and the hydrogen-ions removed by the alkali so that  $[H^+] = 1 \times 10^{-7.07}$  which concentration corresponds roughly with the end-point of the titration. The "acidity" measured by titration methods refers to the total quantity of hydrogen-ions that can be produced from the acid when the ionic equilibrium is continually shifted by the introduction of hydroxyl-ions, and is often called *true* or *total* acidity, but would be more accurately described as *potential* or *titratable* acidity. The potential acidity of such a system as an acid soil may consist partly of undissociated acid dissolved in the soil water, or of acid derived from partly hydrolysed soluble salts such as  $Al_2(SO_4)_3$ , or possibly of a large excess of relatively insoluble or slightly soluble mineral acid or organic acid or even of "adsorbed" acid, such as phosphoric. The presence of "adsorptively unsaturated" compounds, however, such as colloids, in soils affects the hydrogen-ion concentration of the soil solution without necessarily affecting the titratable acidity and the apparently anomalous behaviour of soil extracts made with neutral salt solutions is often attributed to a "selective adsorption" of base.

#### D. Nature of buffer action.

All properties of a solution due to acidity depend really on the particular  $[H^+]$  at the moment\*. Many factors, however, may influence the actual  $[H^+]$ . Thus colloids, acid phosphates, salts of lime, etc. in the soil act as "buffers" or "reaction regulators" in presence of which the soil solution has a strong tendency to maintain its  $[H^+]$  unchanged so that a given amount of acid or base would produce much less alteration in the  $[H^+]$  in presence of a soil than it would in pure aqueous solution. Thus 1 c.c. of N/100 HCl added to one litre of water will decrease the  $-\log [H^+]$  from 7 to 5, the resulting solution being strongly toxic to many bacteria. If on the other hand the same amount of acid be added to a like quantity of water containing 50 or 100 gms. of a soil of  $-\log [H^+] = 7$  the resulting change in  $-\log [H^+]$  is hardly appreciable. This effect is known as *buffer action*, the substances causing the condition being known as *buffers* and the solutions themselves as *buffer solutions*. In the case of pure aqueous solutions the mechanism of buffer action is clear. Thus suppose there is present a mixture of a weak acid,

\* The so-called "neutral salt action" can be neglected in this connection as it in no way invalidates or conflicts with the point of view adopted here. The possibility that it may be a factor to be reckoned with in a consideration of the conditions involved in soil reaction should not however be overlooked. For an account of it of purely physico-chemical interest see Arrhenius (6), Lambie and Lewis (39), and McBain and Coleman (49).

*e.g.* acetic acid, and one of its salts, *e.g.* sodium acetate; the acetic acid is only slightly ionised, so that

$$\frac{[\text{H}^+] \times [\text{Ac}']}{[\text{HAc}]} = K_A = 18.0 \times 10^{-6}.$$

The sodium acetate on the other hand is a strong electrolyte and a very large proportion of it is ionised. This involves an enormous increase in  $[\text{Ac}']$  and in order to keep  $K_A$  of the acetic acid constant  $[\text{H}^+]$  must be proportionately decreased, the result being that such a solution is by no means so sensitive to additions of acid or alkali as is pure water or pure aqueous acetic acid in the absence of the salt. This is clearly seen in the accompanying curve\* (Fig. 1) which represents

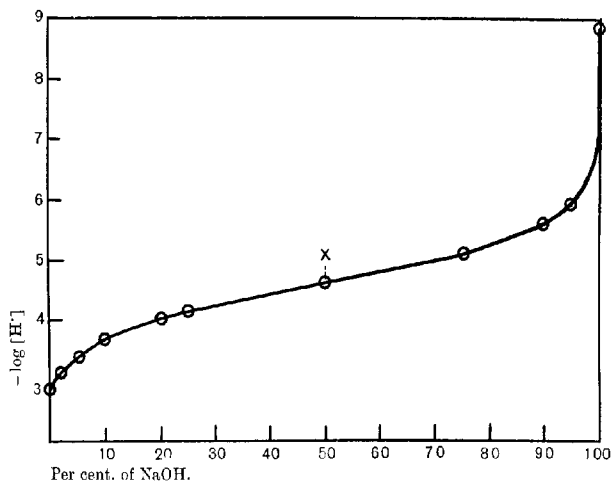


Fig. 1. Neutralisation curve of N/10 Acetic Acid.

the change in  $-\log [\text{H}^+]$  of N/10 acetic acid when it is titrated with N/10 alkali. At either end of the curve when only acetic acid *or* NaAc is present the addition of ten per cent. of NaOH will cause a large alteration in  $-\log [\text{H}^+]$ . At the point X however where the acetate content of the solution consists of 50 per cent. acetic acid and 50 per cent. of Na-acetate the addition of 10 per cent. of NaOH or of HAc will produce only an insignificant effect on the  $-\log [\text{H}^+]$ .

Similar considerations apply to a weak base in the presence of one of its salts.

\* Reproduced from Prideaux (58), p. 228.

The curves shown in Fig. 2\* bring out some features of buffer action that are shown even more strikingly by soil-water mixtures. The curves represent the changes in  $-\log [H']$  when 1 per cent. and 5 per cent. solutions of Witte's peptone are titrated with N/10 lactic acid and N/10 NaOH respectively. An examination of the two curves will show that the amount of buffer action is dependent upon a number of factors among which are:

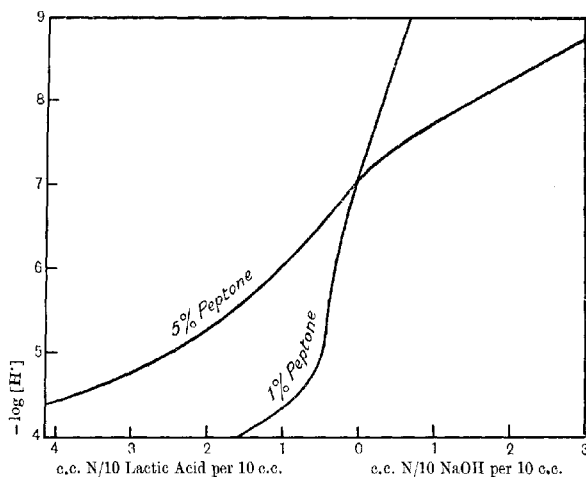


Fig. 2. Titration curves of Peptone Solutions.

1. The *nature* of the constituents as indicated by a comparison of the slopes of the curves in Fig. 2 with the slopes of that in Fig. 1.
2. The concentration of the constituents, the 5 per cent. solution being more resistant to change in  $-\log [H']$ , *i.e.* has greater buffer effect, than the 1 per cent. solution.
3. The buffer effect is not the same at all points of the curve but depends on the region of  $-\log [H']$  at which the buffer action is measured. This last point is important and is brought out better in Fig. 3\*, which is the titration curve of ortho-phosphoric acid. From this curve it will be noticed that there is practically no buffer action at  $-\log [H']$  values of 4.4 and 9.3, but that there is very considerable buffer action in the region of  $-\log [H']$  7.4 to 6.2. This is the region

\* Reproduced from Clark and Lubs' (18) paper.

between which the reaction of normally fertile soils varies, and the possibility that a part may be taken by acid phosphates as reaction regulators in soils has been very largely overlooked. Acid phosphates of course cannot be the only "buffers" in a soil—salts of lime may also act as such although not perhaps in the same region of  $-\log [H']$ . The soil colloids also act as powerful buffers but nothing is known as to the region of  $-\log [H']$  in which they act best. Fig. 2 indicates that Witte's peptone exerts its greatest effect when  $-\log [H']$  is less than 5 but the soil colloids from their very number and diversity may exert considerable effect in all regions of  $-\log [H']$ . Thus 1.67 c.c. N/10  $H_2SO_4$

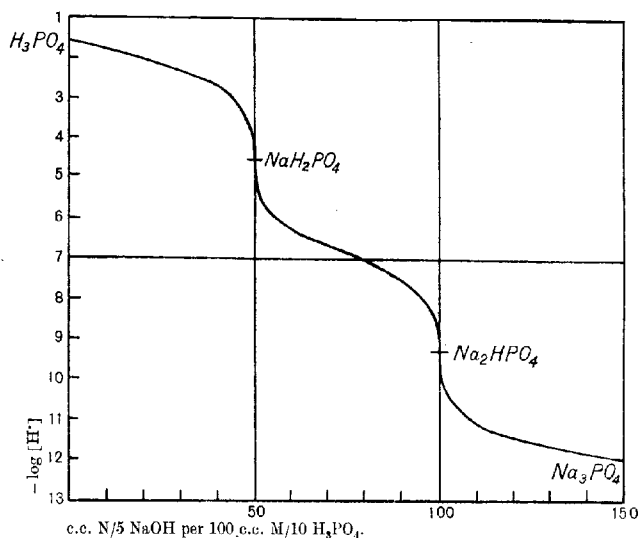


Fig. 3. Titration curve of O-Phosphoric Acid.

added to a mixture of 40 gms. of a slightly acid soil and 80 c.c. of water lowered the  $-\log [H']$  from 6.6 to 5.6; the same amount of acid added to 80 c.c. pure water lowered the  $-\log [H']$  from 6.8 to 2.5. It would appear therefore that the soil complex is well supplied with "buffers" which operate at all regions of  $-\log [H']$ . In view of these facts it is not surprising that although the lime requirements of different soils vary enormously yet the variations of  $-\log [H']$  are confined within much narrower limits. The largest variations of  $-\log [H']$  values of aqueous soil extracts are those given by Sharp and Hoagland (64), 3.7



to 9.7. These are relatively considerable and include extreme cases of acidity and alkalinity. These workers found however that the definitely fertile soils showed strikingly similar reactions: slightly alkaline as indicated by  $-\log[H^+]$  values between 7.04 and 7.52. Such wide variations in  $-\log[H^+]$  as 3.7 to 9.7 must correspond to abnormally large differences in titratable acidity. On the other hand identical or nearly identical values for  $-\log[H^+]$  with different types of soil may exist along with widely different amounts of titratable acidity as found either by the ordinary lime requirement methods or by the colorimetric method described later. It is impossible at present to account completely for the buffer action in soils, and it can be expressed only by the slopes of titration curves.

#### METHODS OF MEASURING SOIL REACTION.

##### *A. Failure of ordinary Titrimetric Methods.*

The older titrimetric methods fail to investigate completely soil reaction, partly because they measure only titratable acidity, and partly because of "adsorptive" and other buffer effects. In all methods based upon extraction of a soil with solutions of salts of weak acids there is considerable obscuring or displacement of the end point of the titration by the buffer action of the salts (Knight<sup>(38)</sup>). It is well known that in mixtures containing weak acids with considerable buffer action near the change point of the indicator even the titratable acid cannot be determined by the ordinary titrimetric methods. With soil extracts the case may be even more complicated: soils have frequently been arranged in the order of their apparent acidities by the use of litmus paper, and Harris attempted to classify soils as "truly acid" and as what Ramann<sup>(59)</sup> called "adsorptively unsaturated" according to whether the extract alone or the moist soil itself were necessary to colour blue litmus paper. Walpole<sup>(77)</sup>, and later Gillespie and Wise<sup>(26)</sup>, showed that both buffer action and time of contact were potent factors in the behaviour of buffered solutions towards litmus paper: N/10,000 HCl in pure water has  $-\log[H^+] = 4.8$  but will produce practically no effect on litmus paper apart from a certain amount of leaching of the dye from the paper after long contact. A standard buffer phosphate solution however of  $-\log[H^+]$  as great as 6.8 reddens blue litmus paper almost instantaneously. The fact that moist soil itself reddens blue litmus paper when its aqueous extract does not implies no necessary difference between "truly acid" and "adsorptively unsaturated" soils, but merely

that the buffer action in aqueous soil extracts in the absence of the solid soil is not sufficient to maintain the  $-\log [H^+]$  unchanged while the reaction of the paper itself was altering. The buffer action of soils is bound up with the solid phase and the reaction of the soil solution is maintained constant only when in contact with the solid phase. It follows therefore that the reaction of soil extracts bears no relation at all to the litmus test when litmus paper is used and both time and buffer factors are left out of account. No difficulty, on the other hand, is experienced in measuring with a fair degree of accuracy the reaction of an aqueous soil extract when suitable precautions are taken and the much more brilliant and sensitive sulphone-phthalein dyes are used in place of litmus.

*B. Study of the Hutchinson-MacLennan Method.*

In order to obtain further information as to the inadequacy of the older methods of determining lime requirements through neglect of possible "adsorptive" factors a study of the Hutchinson-MacLennan method was carried out.

Table II. *Lime requirements\* of the Park Grass Plots as determined by*

Plot No.	(a) Hutchinson's method	(b) Colorimetric method	Ratio $a/b$
<i>A. Low Acidity Group.</i>			
3	0.020 %	0.0064 %	3.125
8	0.055	0.0084	6.55
4-1	0.060	0.0084	7.14
2	0.075	0.0067	11.19
16	0.115	0.0060	19.16
7	0.135	0.0062	21.77
<i>B. Medium Acidity Group.</i>			
1	0.260 %	0.029 %	8.96
13	0.335	0.056	5.98
4-2	0.520	0.129	4.03
<i>C. High Acidity Group.</i>			
10	0.625 %	0.129 %	4.84
11-2	0.705	0.207	3.40
11-1	0.735	0.244	3.01
9 (limed)	0.815	0.295	2.76
9 (unlimed)	0.900	0.317	2.84

\* Expressed as % CaO of air-dried samples.

The lime requirements of many of the Rothamsted grass plots had been determined by this method in 1914, *i.e.* five years after liming and immediately before re-liming. The figures given below by the colorimetric method (see Part II) were obtained in 1919, *i.e.* five years after liming and immediately before re-liming. The figures are not therefore strictly comparable as they were not obtained on the same samples at the same time or by the same worker. It would appear, however, that they should show some sort of rough relationship as the samples were taken at the same point of the liming cycle, provided always that the same function is measured in the two methods. In Table II the two sets of figures are given for comparison.

There is no agreement between the two sets of figures: the results from the first are from three to twenty-two times as great as those from the second, the discrepancy being greatest in the case of the slightly acid plots. Among possible factors operative in causing the discrepancies are the "adsorptive" as distinct from the absorptive properties of the soil. The absorption of lime by an acid soil is probably a twofold process: part of the lime may be used up in neutralising the soil acids, and part may be adsorbed by the soil particles. However accurate therefore the colorimetric and electrometric methods of measuring the  $-\log[H^+]$ , *i.e.* the reaction, of soil-water mixtures may be neither they nor any other known method give exact information as to the actual amount of free acid present in a soil because whenever we attempt to neutralise this acid by adding a base some of the base is always "adsorbed" by the soil itself. We cannot therefore differentiate in practice between the "acidity" and the "lime requirements" of a particular soil: the latter is the more comprehensive term and may be defined as the percentage of lime required to bring the  $-\log[H^+]$  of the soil to 7.07, always bearing in mind that this lime is utilised partly in neutralising soil acids and partly in being "adsorbed" by the soil itself. The amount of lime adsorbed can be expressed by the following general equation

$$\frac{y}{m} = kC^{\frac{1}{p}},$$

in which  $y$  is the amount of lime adsorbed by  $m$  gms. of soil,  $C$  is the final concentration of lime in the liquid phase, while  $k$  and  $p$  are constants.

When  $-\log H = 7.07$ ,  $C$  is in the neighbourhood of zero, so that perfectly consistent and comparable results should be obtained by the colorimetric method provided the method is experimentally sound. In Hutchinson's method, however,  $C$  is always some positive quantity,

*i.e.* it is the amount of lime left in the solution after shaking with the soil and it should therefore follow that the amount of lime adsorbed is greater the greater  $C$  is. In other words since  $N/50 \text{ CaH}_2(\text{CO}_3)_2$  is used for all soils, then the smaller the acidity the larger will  $C$  be and therefore the larger relatively will  $y/m$  be. It follows that the error inherent in this method for a given type of soil will be greater the smaller the acidity of the soil examined. This is supported by the fact already noted in Table V that the discrepancy between the two methods when applied to the same type of soil is greater the less the acidity. Moreover in no sense can the soil be said to have been neutralised by the lime absorbed from the bicarbonate solution even after equilibrium has been attained, because owing to the large excess of  $\text{CO}_2$  the  $-\log[\text{H}^+]$  of the resulting solution is in the neighbourhood of 6.0 and one cannot consider a soil to have been neutralised when in equilibrium with an acid solution. If these considerations are valid then it would follow that a neutral soil and, with heavier types of soil, even soils with a definite  $\text{CaCO}_3$  content may show positive lime requirements with the Hutchinson method but none by the colorimetric method. This is denied by Hutchinson and MacLennan who say "The method possesses the advantage over several others suggested in that it indicates no absorption in the case of neutral soils." Yet elsewhere in a Table they give examples of soils possessing appreciable  $\text{CaCO}_3$  contents and yet having considerable lime requirements\*.

	Rothamsted	Chelsea	Devon	Millbrook	Geescroft	Metchley	Harpen Adams	Woburn	Craikstone	Leeds 11
$\text{O}_2$ present %	2.660	0.890	0.003	0.035	0.005	0.097	0.005	0.003	Nil	Nil
$\text{O}_2$ required %	Nil	Nil	0.015	0.032	0.100	0.117	0.135	0.260	0.430	0.470

Wild (81) in a study of some New Zealand soils noticed similar phenomena. A soil containing 10 per cent.  $\text{CaCO}_3$  showed a small, though positive, lime requirement by Hutchinson's method. Further, as indicated by the above general formula, the adsorption is of the nature of a balanced reaction; the quantity adsorbed is in equilibrium with the final concentration,  $C$ , of the surrounding solution. If the bicarbonate solution in equilibrium with the soil is replaced by one of a greater concentration than  $C$  the soil should give up lime to this solution until a fresh equilibrium is attained. Wild mentions a soil containing "a large excess of calcium carbonate" that gave up lime to the  $N/50$  bicarbonate

\* It is fair to point out that the authors suggest another explanation, an explanation that may hold in some cases. But they suggest it only and adduce no evidence to show that it applies to the examples given in the Table.

solution used: other instances are also recorded. Hutchinson and MacLennan realised the effect of the *initial* concentration of bicarbonate solution:

Initial concentration of solution	N/50	N/75	N/100
Absorption (as percentage of soil)	0.272	0.265	0.210

But they associate the initial concentration only with the time taken for attainment of equilibrium and do not discuss the effect of the final (equilibrium) concentration.

Table III.

$y/m$ = mgs. CaO absorbed per													
10 gms. soil	...	...	1.54	1.82	1.98	3.25	3.49	3.66	3.69	3.71	3.96	4.52	4.69
$C$ = mgs. CaO left in 100 c.c.													
solution	...	...	10.37	14.16	11.06	6.02	6.805	16.40	21.80	6.10	7.39	28.57	13.58
$y/m$ = mgs. CaO absorbed per													
10 gms. soil	...	...	5.055	5.26	5.376	6.91	7.00	8.495	9.31	11.20	11.55	57.67	
$C$ = mgs. CaO left in 100 c.c.													
solution	...	...	12.90	14.26	14.06	23.50	14.00	22.25	28.40	43.26	57.80	38.10	

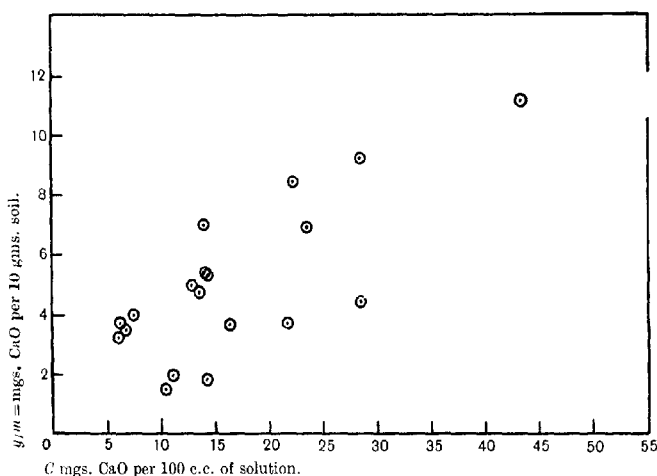


Fig. 4. Absorption of calcium bicarbonate by soil (3 mm. sample).

Wild found that the initial concentration of the bicarbonate solution affected not only the time required for apparent equilibrium to be attained but also the amount of lime absorbed.

If adsorption occurs then on plotting the amount of lime adsorbed by the soil against *final* equilibrium concentration of lime in the bicar-

bonate solution, *i.e.* plotting  $y/m$  of the general equation against  $C$ , a particular type of curve should be obtained expressible by the general equation when suitable values for the constants  $k$  and  $p$  are chosen. No such curve could be obtained from Wild's data as he gives only initial and not final concentrations. An attempt was therefore made to see whether the effect of  $C$  on  $y/m$  followed the usual adsorption equation. Approximately N/50  $\text{CaH}_2(\text{CO}_3)_2$  solution prepared in the usual way was diluted with distilled water to make a series of solutions of N/50, N/75, N/100, N/125, N/150, N/175, and N/200. Samples of soil (Grass Plot 19, air-dried 3 mm. sample) varying from 5 gms. to 30 gms. were shaken with varying volumes of the above solutions in an end-over-end shaker for three hours. Aliquot parts of the rapidly filtered extracts were then titrated with standard  $\text{H}_2\text{SO}_4$ , N/10 acid being used for the stronger and N/50 acid for the more dilute extracts. The values of  $y/m$  (= mgs.  $\text{CaO}$  absorbed per 10 gms. of soil) and  $C$  (= mgs.  $\text{CaO}$  remaining per 100 c.c. of solution) are given in Table III and plotted in Fig. 4. These experimental points do not lie even approximately on a curve, but their very irregularity and the fact that some of the points were really duplicates and should therefore have coincided suggests some disturbing factor. The irregularities were traced to the unequal state of division of the soil samples. Adsorption is essentially a surface phenomenon and the amount of lime adsorbed should be in direct proportion to the amount of surface exposed. The 3 mm. sample would consist of particles of all sizes less than 3 mm. and not only is it certain to lack uniformity but the effective surface increases irregularly by the breaking up of the particles during the shaking. This would account for the anomalies suggested by Fig. 4 especially if the lime could only get at the interior surfaces within the larger crumbs by a slow process of diffusion. To test this point a series of determinations was carried out on soil from Grass Plot 18 which had been ground up in a mortar with a wooden pestle until practically the whole of it passed through a sieve with square meshes of 100 to the linear inch. The small percentage which would not pass through, consisting mainly of silica, was thoroughly mixed with the rest so as to avoid any effect due to fractionation. The determinations were carried out on this finely ground sample on the same day and the results are given in Table IV and Fig. 5.

In this series the points lie very fairly about a parabolic curve typical of adsorption isotherms and which is expressed quite well by the equation

$$y/m = kC^{1/p},$$

when  $k = 5.13$  and  $1/p = 0.475$ ,

i.e.  $y/m = 5.13C^{0.475}$ .

Moreover by a simple transformation we get

$$\log (y/m) = 1/p \log C + \log k,$$

Table IV.

$y/m$ = mgs. CaO absorbed per 10 gms. soil	33.60	* (22.68 22.26	17.22	13.50	7.89	* (4.09 4.525
$C$ = mgs. CaO remaining per 100 c.c. solution	50.96	* (21.28 21.42	12.74	5.74	3.36	* (0.980 0.420

\* Duplicates.

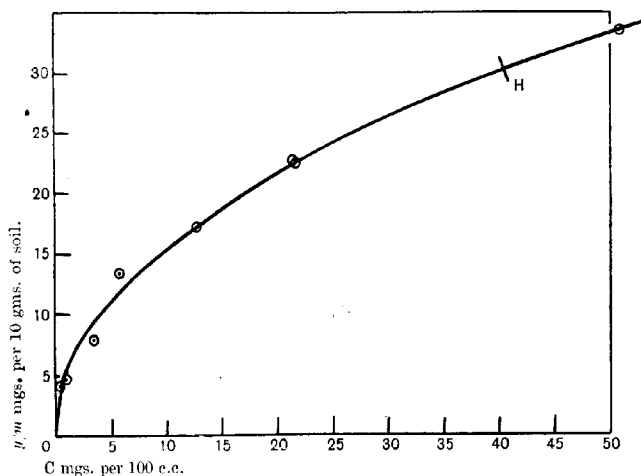


Fig. 5. Absorption of calcium bicarbonate by soil (finely ground sample).

and by plotting  $\log (y/m)$  against  $\log C$  the points obtained should lie approximately on a straight line. It will be seen from Fig. 6 that they lie distributed near a straight line with a very fair degree of approximation\*. There would appear to be little doubt therefore that the lime requirement of a soil is the sum of two quantities: (1) the lime required to neutralise soil acids, and (2) the lime actually adsorbed by the soil complex and it is impossible at present to differentiate quantitatively

\* It is from this approximately straight log. curve that we can obtain values for the constants  $k$  and  $1/p$  with a very fair degree of approximation since when  $\log C = 0$  the intercept cut off by the curve from the  $y$  axis will equal  $\log k$  while the slope of the curve will equal  $1/p$ .

between these. It follows that equivalent quantities of different bases will not necessarily be equally effective in neutralising a soil unless we assume that they are equally adsorbed by the soil, which does not appear to be the case. Without further evidence therefore it is not legitimate to use baryta to neutralise a soil and then to calculate the result in terms of lime. (See Part II, p. 59.)

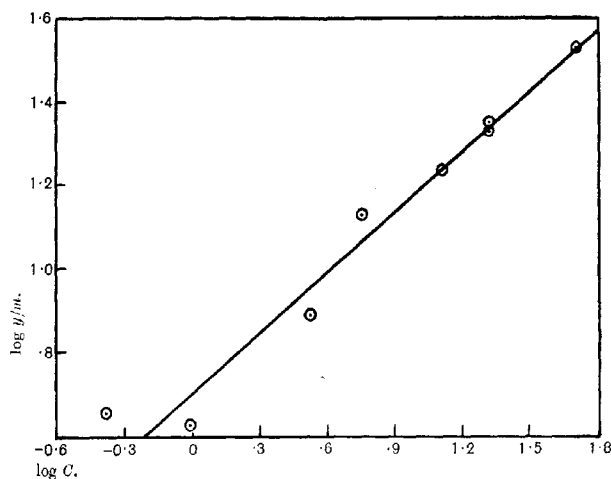


Fig. 6.

It is obvious that comparable results for the lime requirements of different soils cannot be obtained even approximately by the Hutchinson-MacLennan method as at present carried out. In the case of Plot 18 the lime requirement of the finely ground sample as determined by the Hutchinson method using 250 c.c. N/50  $\text{CaH}_2(\text{CO}_3)_2$  and 15 gms. of the finely ground soil (the usual proportion) should be about 0.300 per cent. and is indicated on the curve (Fig. 5) by the point *H*; it was found actually to be 0.302 per cent. But, as the curve indicates, the apparent lime requirement increases with *C* and moreover the shape and slope of the curves are very different for different soils, the curve being very flat and close to the *C* axis for light sandy soils and very steep (much steeper than the one shown in Fig. 5) for heavy clay soils. To obtain comparable results therefore by Hutchinson's method an experimental curve of the type shown (or at any rate a considerable region of it round about the point *H*) should always be drawn. A lime requirement corre-



sponding to a fixed and constant final equilibrium concentration  $C$  could then always be obtained by interpolation and provided that the correct  $C$  was chosen as a reference point it is possible that fairly accurate and comparable results might be obtained\*. The correct  $C$  could, however, be chosen only as the result of a large number of field trials on many different types of soil, and would not necessarily be the same with different soil types or with different systems of manuring. Moreover in carrying out the determinations finely ground samples should be employed owing to the impossibility otherwise of attaining equilibrium in a reasonable number of hours, and moreover because ground and unground soils possess different lime requirements. Thus two lots of 15 gms. each of the 3 mm. sample and two lots of 15 gms. each of the 100 mesh per inch sample from Plot 5-2, were shaken with 250 c.c. N/50  $\text{CaH}_2(\text{CO}_3)_2$  for three hours. They were then filtered and, 100 c.c. of each titrated with N/10  $\text{H}_2\text{SO}_4$ . The results were as follows:

	Original N/50 $\text{CaH}_2(\text{CO}_3)_2$		Filtrate from 3 mm. sample		Filtrate from 100 mesh per in. sample	
100 c.c. required c.c. N/10 $\text{H}_2\text{SO}_4$	(a) 21.98	21.98 c.c.	18.33	17.67 c.c.	16.98	16.845 c.c.
	(b) 21.98		17.01		16.71	
Lime requirement expressed as gm. CaO per 100 gms. air-dried soil				0.2016		0.2354

Thus in the case of the finely ground samples not only did the duplicates agree much better but the lime requirement was 16.7 per cent. higher than that of the 3 mm. sample.

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\* The above argument has been expressed in terms of the selective adsorption theory because this theory is widely accepted as satisfactory. Exactly similar results would be expected whether the lime is "adsorbed" on the surface of the soil particles or chemically precipitated by interaction with *e.g.* alumina to form calcium aluminate; the form of curve in the latter case would be determined by solubility and hydrolysis relationships. See above, p. 22 and also Meyerhoffer's (51, 52, 53) work on the solubility relationships in reciprocal salt pairs.

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## STUDIES ON SOIL REACTION. II.

### THE COLORIMETRIC DETERMINATION OF THE HYDROGEN ION CONCENTRATION IN SOILS AND AQUEOUS SOIL EXTRACTS. (PRELIMINARY COMMUNICATION.)

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THE methods used for the determination of  $-\log [H^+]$  fall naturally into two groups:

- (a) Electrometric, and
- (b) Colorimetric methods.

Electrometric methods were first introduced into analytical practice in 1897 by Böttger<sup>(1)</sup> who determined the neutral point in titrating acids with alkalis by using a gas chain; subsequent improvements were made by Hildebrand<sup>(16)</sup>, Cumming and Gilchrist<sup>(10)</sup>, Hasselbalch<sup>(15)</sup>, W. M. Clark<sup>(6)</sup>, Michaelis<sup>(19)</sup>, Walpole<sup>(28,29)</sup> and others. The method has been applied to the measurement of  $[H^+]$  of biological fluids with considerable success\*. It was first applied to the measurement of  $[H^+]$  of soil suspensions by G. Fischer<sup>(12)</sup> in Germany in 1914 and subsequently in America by Sharp and Hoagland<sup>(23)</sup>, and by Gillespie<sup>(13,14)</sup> and his co-workers.

The colorimetric method was introduced by Sørensen<sup>(24,25)</sup> in 1909, and was improved and applied to biological fluids by Sørensen<sup>(26)</sup>, by Palitzsch<sup>(21)</sup>, and in this country by Walpole<sup>(28)</sup>. Basing their work on the same principles but using a different set of indicators and of buffer mixtures Clark and Lubs<sup>(7,8)</sup> have further improved the method. Gillespie and his co-workers<sup>(13,14)</sup> applied both methods to soils with excellent agreement in spite of the fact that they employed aqueous soil extracts in the colorimetric method, but mixtures of soil and water in the electrometric method, soil extracts alone being unsuitable in the latter case as they are relatively poor in buffer action. Further the  $NO_3^-$  ions were reduced to  $NH_3$  by the hydrogen of the electrode, thus

\* See detailed account of whole method in Michaelis, L., *Die Wasserstoffionen-Konzentration*, J. Springer, Berlin, 1914.

rendering a constant potential impossible of attainment, and in some cases even changing the reaction of the whole fluid to indicators. This is the only systematic comparison so far made between the two methods as applied to soils and it deserves repetition on account of its fundamental importance\*.

#### THE COLORIMETRIC METHOD.

The colorimetric method of determining hydrogen-ion concentration depends on the fact that for every indicator there is a particular zone of  $[H^+]$  or of  $-\log [H^+]$  within which its colour changes but gradually. A large number of indicators are known each having its own particular zone of change which differs from that of most other indicators. Thus methyl red changes its colour gradually from yellow through brown to red within the zone of  $-\log [H^+]$  of 6.0 to 4.4; phenol phthalein changes colour between  $-\log [H^+]$  10 to 8.5, litmus between 8 and 5, methyl orange 4.0 to 3.0. Moreover many of these ranges overlap so that the tints produced on a particular indicator by a particular solution, *A*, allow of direct comparison of its  $-\log [H^+]$  with reference to that of a standard solution *B*. At certain points where the ranges of two or more indicators overlap the results can be checked by using more than one indicator for the determination of the same  $-\log [H^+]$ . The method is not absolute; it does not really measure the  $-\log [H^+]$  of a solution but only shows that this function is identical with that of a particular standard. Ultimately the absolute  $-\log [H^+]$  of the standards must be determined by the more fundamental electrometric method. If the standard solutions are strongly "buffered" by the presence of reaction regulators they will maintain their  $-\log [H^+]$  unchanged for considerable periods since small quantities of impurities from the air, glass, or slight mould growth, etc., have but little effect. Further, the standard buffer solutions are generally easily prepared and once made up and their  $-\log [H^+]$  measured electrometrically they can easily be renewed without making fresh electrometric determinations. Where a

\* Rice and Oslugi(22) showed that the inversion of sucrose by soils is invariably greater than that of soil extracts, results which apparently militate against the validity of the electrometric method. These authors however did not prove that nothing else was present in soils capable of inverting sucrose except hydrogen-ions so that the validity of their method rests upon a somewhat precarious foundation. On the other hand the electrometric method would appear to measure only the  $[H^+]$  of the liquid aqueous phase, the work of Bovie (3) indicating that any hydrogen-ions adsorbed on the surface of the solid phase play no part in the electrical potential generated. This point however requires further work as it would appear to present difficulties.

considerable degree of accuracy is required however the standard buffer solutions should be examined electrometrically at frequent intervals, but for routine work or where fairly accurate comparisons are required without a high degree of accuracy in the absolute values the solutions need not necessarily be checked electrometrically but should be renewed at frequent intervals and the new ones checked against the old.

The essentials of the method are: first a set of indicators that can be used to cover the required ranges of  $-\log [H^+]$  and, secondly, a set of standard buffer solutions for use within these ranges.

The standard buffer solutions are generally mixtures of some acid and its alkali salt. Clark and Lubs<sup>(7,8)</sup> standardised the following series:

Acid potassium phthalate and hydrochloric acid.

Acid potassium phthalate and sodium hydroxide.

Acid potassium phosphate and sodium hydroxide.

Boric acid, potassium chloride and sodium hydroxide.

This series possibly has certain advantages over the older ones of Sørensen<sup>(24,25,26)</sup>, Palitzsch<sup>(21)</sup> and Walpole<sup>(28)</sup>. The simplicity and ease of preparation is a real advantage in ordinary laboratory routine. Only four substances are involved to cover a range of  $-\log [H^+]$  from 2.2 to 10.0. The technique of the preparation is simple especially in comparison with that of the acetate mixtures of Walpole<sup>(29)\*</sup>. Both acid potassium phthalate and acid potassium phosphate have no water of crystallisation and can therefore be oven-dried at 110° C. And only one alkaline substance is involved and therefore only one that need be protected against the CO<sub>2</sub> of the air.

As will be seen from Fig. 1 the phthalate and phosphate curves and the phosphate and borate curves overlap, hence at  $-\log [H^+] = 5.8, 6.0$  and  $6.2$ , and  $7.8$  and  $8.0$  we get two series of solutions of the same  $-\log [H^+]$  which serve as very useful checks on the accuracy with which the standard solutions have been made up.

Acid potassium phthalate is also excellent for standardising the sodium hydroxide†, and hence indirectly the hydrochloric acid which can further be checked by precipitation as AgCl. The potassium phosphate, potassium chloride and boric acid can all be accurately weighed.

\* In this case in particular the elaborate method of purification and the difficulty in really preventing mould growth are serious troubles; in the latter respect the glyceoll mixtures are no better.

† Acid potassium phthalate is now on the market for this purpose, but in spite of its high price should *always* be recrystallised once before use at a temperature above 20° C. Below this temperature the so-called tri-phthalate separates out.

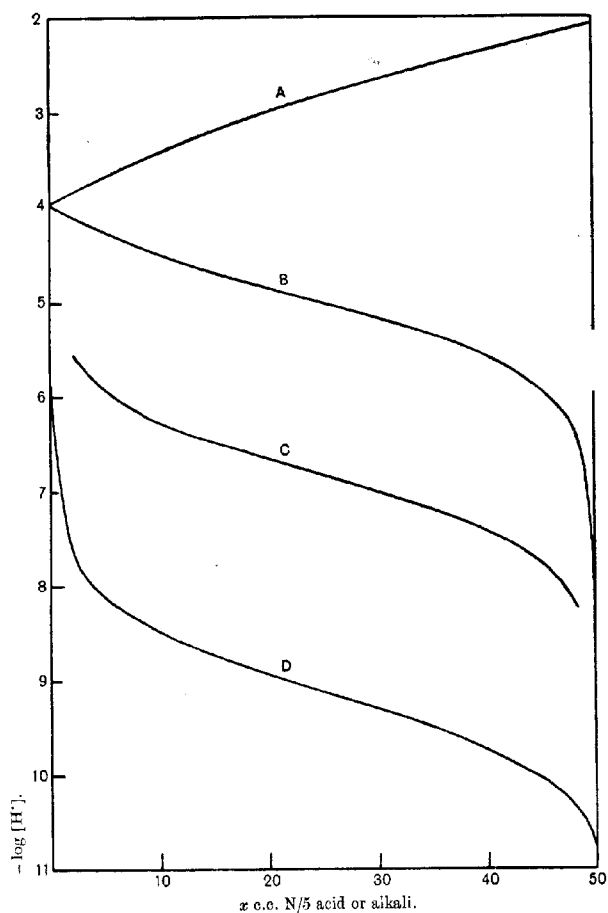


Fig. 1. Showing variation in  $-\log[H^+]$  of standard buffer solutions with amounts of HCl or NaOH added.

- A. 50 c.c.  $M/5$  H-K-Phthalate +  $x$  c.c.  $M/5$  HCl, the whole diluted to 200 c.c.
- B. 50 c.c.  $M/5$  H-K-Phthalate +  $x$  c.c.  $M/5$  NaOH, the whole diluted to 200 c.c.
- C. 50 c.c.  $M/5$   $KH_2PO_4$  +  $x$  c.c.  $M/5$  NaOH, the whole diluted to 200 c.c.
- D. 50 c.c.  $M/5$  ( $H_3BO_3 + KCl$ ) +  $x$  c.c.  $M/5$  NaOH, the whole diluted to 200 c.c.

A slight disadvantage this series possesses in comparison with the older ones: in standard buffer mixtures the  $-\log [H^+]$  depends entirely on the ratio of the acid to base. Sørensen used  $Na_2HPO_4$  as the base in his mixtures and Palitzsch used  $Na_2B_4O_7$  in his borate mixtures. In Clark and Lubs' series the base is the much stronger  $NaOH$ , so that very accurate standardisation is necessary to avoid serious errors at the alkaline end of the series.

#### PREPARATION OF STANDARD BUFFER SOLUTIONS.

The mode of preparation of the standard solutions was essentially that given by Clark and Lubs(7,8). The following stock solutions were used:

M/5  $HCl$ , M/5  $KH_2PO_4$ , M/5  $HKC_8H_4O_4$ , M/5  $H_3BO_3$  with M/5  $KCl$ , and M/5  $NaOH$ .

The water used throughout the investigation was "conductivity" water made according to the directions of Bourdillon(2)\*. All weights, burettes, pipettes and measuring flasks were calibrated and in all cases the purest salts obtainable were purchased. The  $HCl$  was purified by diluting pure concentrated  $HCl$  to 20 per cent. and distilling. An M/5 stock solution was made up and standardised by the  $AgCl$  method and checked with the standard soda which had been standardised by Dodge's method described below.

The potassium chloride was recrystallised three times from "conductivity" water and dried in a hot air oven at  $120^\circ C$ . for two days. An M/5 solution contains 14.912 gms.  $KCl$  per litre.

Acid potassium phthalate ( $HKC_8H_4O_4$ ) was recrystallised three times from "conductivity" water, the crystals being drained with suction on a Buchner funnel after each crystallisation. The salt was dried by heating in an air oven at  $110-115^\circ C$ . until after cooling in a desiccator over  $CaCl_2$  the weight remained constant. An M/5 solution contains 40.828 gms.  $HKC_8H_4O_4$  per litre.

Acid potassium phosphate ( $KH_2PO_4$ ) was recrystallised three times

\* The "conductivity" water made by the Bourdillon still is of exceptionally good quality. According to Bourdillon seven or eight litres can be obtained by a single distillation from ordinary tap water of a conductivity of less than 0.2 gemmho ( $=0.2 \times 10^{-6}$  reciprocal ohms). The present writer was not in a position to measure its conductivity but always measured the  $-\log [H^+]$  colorimetrically of every fresh supply, and generally daily. This was rarely found to be less than 6.8 if the measurement was carried out as rapidly as possible. The water however was so sensitive to the action of  $CO_2$  of the air that the  $-\log [H^+]$  fell rapidly on exposure, reaching in the course of a few seconds the value of about 6 or less.



from conductivity water, the crystals being drained with suction on a Buchner funnel after each crystallisation and dried by heating in an air oven at 110–115° C. to constant weight. An M/5 solution contains 27.232 gms.  $\text{KH}_2\text{PO}_4$  per litre. (As a rough test of purity the solution should be distinctly red to methyl red and distinctly blue to brom phenol blue.)

Boric acid was recrystallised three times from “conductivity” water. It was air-dried in thin layers between filter paper (as it loses “water of constitution” above 50° C.) and finally dried to constant weight in thin layers in a desiccator over  $\text{CaCl}_2$  for several weeks. An M/5 solution contains 12.4048 gms. boric acid and also 14.912 gms. KCl. The borate curve overlaps the phosphate curve and the object of having the solution M/5 to KCl as well as to boric acid is to make the salt content comparable with the salt content of the phosphate solutions as it is well known that neutral salts themselves have a distinct, though small, effect on the colours of indicators.

The sodium hydroxide is the most difficult of the series to obtain pure. It was made as follows: 100 gms. of the purest NaOH were dissolved in 100 c.c. “conductivity” water in a conical flask. The mouth of the flask was loosely corked with a cork covered with tin foil, and allowed to stand over night. Most of the carbonate settles and the solution was filtered as follows: a “hardened” filter paper on a Buchner funnel was treated with a warm 50 per cent. solution of NaOH for a few minutes. The NaOH solution was decanted off and the paper washed first with absolute alcohol, then with diluted alcohol, and finally with conductivity water. At the end of the process gentle suction was applied until most of the water had evaporated but not to the extent that the paper began to curl. The concentrated alkali was then poured upon the middle of the paper, spread with a glass rod so that the paper under gentle suction adhered well to the funnel and the solution was then drawn through by increasing the suction. The clear filtrate was diluted (after rough calculation) to a strength of about N, 10 c.c. were withdrawn and titrated with standard acid and from this preliminary standardisation the dilution required to bring the concentration down to M/5 was calculated. This dilution was made and, with as little exposure as possible, the whole was transferred to a large bottle\* fitted with a calibrated burette and soda lime guard tubes.

\* This bottle should either be an *old* one, preferably one that has been used for years for storing soda; or if new should be thickly coated on the inside with paraffin wax, about 1 lb. to a five litre bottle.

The soda was then accurately standardised by Dodge's<sup>(11)</sup> method, using the purified acid potassium phthalate. Several portions of  $\text{KHC}_8\text{H}_4\text{O}_4$  of about 1.6 gms. were weighed out and each portion was dissolved in about 20 c.c. conductivity water to which was added four drops of phenol phthalein solution. A stream of  $\text{CO}_2$ -free air was drawn through the solutions (or the  $\text{CO}_2$  can be boiled off) which were then titrated with the soda till a faint but distinct and permanent pink colour developed.

Five or six litres of each of these M/5 stock solutions were prepared and the phosphate and phthalate solutions were protected against mould growth by the addition of a little calomel.

From these stock solutions the standard buffer solutions were made up according to the table below. 200 c.c. of each solution were made and kept in a stoppered bottle. In the case of the borate mixtures the bottles were paraffined on the inside.

Table I. *Composition of mixtures giving  $-\log [\text{H}']$  values at intervals of 0.2.*

A. <i>Phthalate-HCl mixtures</i> <sup>1</sup> .										
$-\log [\text{H}']$	2.20	2.40	2.60	2.80	3.00	3.20	3.40	3.60	3.80	
x c.c. M/5 HCl	46.70	39.60	32.95	26.47	20.32	14.70	9.90	5.97	2.63	
B. <i>Phthalate-NaOH mixtures</i> <sup>2</sup> .										
$-\log [\text{H}']$	4.00	4.20	4.40	4.60	4.80	5.00	5.20	5.40	5.60	5.80
x c.c. M/5 NaOH	0.40	3.70	7.50	12.15	17.70	23.85	29.95	35.45	39.85	43.00
									45.45	47.00
C. <i>K-H<sub>2</sub>Phosphate-NaOH mixtures</i> <sup>3</sup> .										
$-\log [\text{H}']$	5.80	6.00	6.20	6.40	6.60	6.80	7.00	7.20	7.40	7.60
x c.c. M/5 NaOH	3.72	5.70	8.60	12.60	17.80	23.65	29.63	35.00	39.50	42.80
									45.20	46.80
D. <i>Boric Acid, KCl-NaOH mixtures</i> <sup>4</sup> .										
$-\log [\text{H}']$	7.80	8.00	8.20	8.40	8.60	8.80	9.00	9.20	9.40	9.60
x c.c. M/5 NaOH	2.61	3.97	5.90	8.50	12.00	16.30	21.30	26.70	32.00	36.85
									40.80	

<sup>1</sup> 50 c.c. M/5 H-K-Phthalate + x c.c. M/5 HCl, the whole diluted to 200 c.c.

<sup>2</sup> 50 c.c. M/5 H-K-Phthalate + x c.c. M/5 NaOH, the whole diluted to 200 c.c.

<sup>3</sup> 50 c.c. M/5  $\text{KH}_2\text{PO}_4$  + x c.c. M/5 NaOH, the whole diluted to 200 c.c.

<sup>4</sup> 50 c.c. M/5 ( $\text{H}_3\text{BO}_3 + \text{KCl}$ ) + x c.c. M/5 NaOH, the whole diluted to 200 c.c.

The consistency of the series was always tested by checking the phthalate-NaOH mixtures of  $-\log [\text{H}']$  5.8, 6.0 and 6.2 against the phosphate-NaOH mixtures of the same  $-\log [\text{H}']$  using brom cresol purple as indicator, and the phosphate-NaOH mixtures of  $-\log [\text{H}']$  7.8 and 8.0 against the boric acid-NaOH mixtures of the same  $-\log [\text{H}']$  using phenol red as indicator.

The standard buffer solutions will not keep indefinitely but need not be made up weekly, as recommended by Clark and Lubs(8), if a trace of calomel is added. Such solutions keep easily for considerable periods although it is inadvisable in the absence of electrometric control to keep them in use for longer than a month or six weeks. In every case newly made up standards should be compared colorimetrically with those about to be discarded in order to see whether any appreciable alteration in  $-\log [H^+]$  has occurred during the period of use. The writer was never able to detect any such change.

*Indicators used.*

Of the many indicators studied by Clark and Lubs the following series\* was recommended by them to cover the whole range of  $-\log [H^+]$  from 1.2 to 9.8:

Table II. *List of indicators.*

Indicator	Common name	Concentration %	Colour change	Range of $-\log [H^+]$
✓ Thymol sulphone phthalein (acid range) ... ..	Thymol blue	0.04	Red-yellow	1.2-2.8
✓ Tetra-bromo-phenol sulphone phthalein ... ..	Brom phenol blue	0.04	Yellow-blue	3.0-4.6
✓ O-carboxy benzene-azo-dimethyl aniline ... ..	Methyl red	0.02	Red-yellow	4.4-6.0
O-carboxy benzene-azo-di propyl aniline ... ..	Propyl red	0.02	Red-yellow	4.8-6.4
✓ Di-bromo-o-cresol sulphone phthalein ... ..	Brom cresol purple	0.04	Yellow-purple	5.2-6.8
✓ Di-bromo-thymol-sulphone phthalein ... ..	Brom thymol blue	0.04	Yellow-blue	6.0-7.6
✓ Phenol sulphone phthalein ...	Phenol red	0.02	Yellow-red	6.8-8.4
O-cresol sulphone phthalein...	Cresol red	0.02	Yellow-red	7.2-8.8
✓ Thymol sulphone phthalein (alkaline range) ... ..	Thymol blue	0.04	Yellow-blue	8.0-9.6
O-cresol phthalcin ... ..	Cresol phthalein	0.02	Colourless-red	8.2-9.8

Of this series propyl red, cresol red and cresol phthalein are not absolutely necessary as the remaining six form a complete overlapping series in themselves. In the present investigation therefore the former were not used.

\* For their preparation and purification see Lubs and Clark(18), and for a critical study of their usefulness as indicators see Clark and Lubs(8). These indicators are now on the market.

The methyl red solution was made by dissolving 0.1 gm.\* in 300 c.c. re-distilled alcohol and diluting to 500 c.c. with conductivity water.

The other indicators were all used in aqueous solution as the monosodium salts: brom cresol purple should dissolve easily in 1.5 equivalents of soda, the others in 1.1 equivalents. Stock solutions were prepared of 0.6 per cent. strength in the case of phenol red and of 1.2 per cent. strength in the case of the others. Such stock solutions are now on the market, but it is always best to make up the solutions oneself. In fact for accurate work it is very necessary to eliminate every trace of the acetic acid used in the preparation or purification, otherwise the indicator requires more than 1.1 equivalents† of base for its solution, and further the sodium acetate produced (different in amount according to the degree of purity of the material) may exert appreciable and variable "salt action‡" on the colour changes produced by the various standard and other solutions to be tested. By making up the stock solutions oneself one has a useful check on the purity of the indicators supplied.

Before using, 10 c.c. of each of these solutions (except of course the methyl red which is ready for use) should be diluted to 300 c.c. with water to make the solutions used in the tests.

Of the six indicators used in this investigation two, *viz.* brom phenol blue and brom phenol purple, show considerable dichromatism under ordinary conditions. With suitable precautions this does not present any difficulties when the indicators are used in daylight, but real difficulty occurs in using them for colorimetric comparisons in ordinary

\* The methyl red of commerce sometimes contains acetate from which it must be freed as it is one of those indicators the colour change of which is affected by "neutral salt action." The methyl red was recrystallised from toluene.

† 1.5 equivalents in the case of brom cresol purple.

‡ In determining  $-\log[H^+]$  electrometrically and colorimetrically differences have been frequently observed between the two methods which have been traced to the influence of proteins or of neutral salts on the colour changes of the indicators. Some indicators are to some extent precipitated or adsorbed by protein or colloid bodies, *e.g.* congo red by the casein of milk. Such an effect will result not only in a decrease of intensity of colour but in the case of dichromatic indicators and in all cases of turbid or slightly coloured media an alteration in quality of the colour as well. This is the so-called *protein effect*. Many salts exert a similar effect on indicators which has been discussed at some length by Michaelis and Rona (20), by Sørensen (24, 25), and especially by Sørensen and Palitzsch (27). The mechanism of this *salt action* is not understood and in general such salt errors cannot be eliminated although in certain specific cases they can be estimated and empirical corrections applied as in the work of Sørensen and Palitzsch (27) on sea water. According to Clark and Lubs (8) the sulphone phthalein indicators have very small salt errors. See also Brightman, Meacham and Acree (4).

electric light. Daylight is rich, while ordinary electric light is poor, in blue rays, so that brom phenol blue and brom cresol purple will appear blue in daylight and reddish in the electric light, thereby introducing difficulties especially in the case of turbid or slightly coloured solutions. Most of the determinations recorded in this paper were carried out in daylight. All the indicators of the sulphone phthalein series exhibit dichromatism to a certain extent and under certain conditions. Also thymol blue and brom thymol blue, changing as they do from yellow to blue, show much greater contrasts when viewed in light rich in blue rays, so that when any of these four indicators had to be used in artificial light the light of the mercury vapour lamp was invariably employed.

On the other hand methyl red shows up better in the ordinary electric light (rich in red) than in the light of the mercury lamp (poor in red) and phenol red has its incipient dichromatism brought out and made more pronounced in the light of the mercury lamp which was not therefore used with these two indicators.

#### METHOD OF USING COLOUR STANDARDS.

The actual colour standards were made up as follows: a series of test tubes 4 inches by  $\frac{3}{8}$  inch were selected so that the internal diameters were exactly the same throughout the series. 10 c.c. of standard buffer solution were transferred to each test tube, four drops of the indicator solution added to each\*, the test tubes were carefully shaken with a rotary motion, closed by rubber caps and stored in a large test tube rack. These solutions have to be renewed at least once a week as the indicators at this small concentration tend to fade in sunlight especially at the paler end of each series of standards, the yellow and brownish ends of the methyl red series being particularly bad in this respect. In this case also each freshly made series was compared colorimetrically with the previous one.

Biological fluids and soil extracts present two main difficulties: the interfering effects of the turbidity and of the natural colour of the fluid. These were overcome by a slight modification of Walpole's(28) method. A small white test tube rack was made to hold six test tubes in two rows of three each. A wooden partition separated each pair from the others in such a way that no light could pass through except actually through the test tubes and the coloured solutions contained

\* In the case of methyl red six drops were used as the size of the drop in this case was much smaller than with the other indicator solutions owing to the reduced surface tension due to the high alcohol content.

in them. The test tubes *A*, *B* and *C* contained 10 c.c. each of the solution whose  $-\log [H^+]$  was to be measured and which was generally turbid and often slightly yellowish or brownish in colour; while *E* contained 10 c.c. of distilled water. *D* and *F* were two of the colour standards with  $-\log [H^+]$  differing by 0.2. Four drops of indicator solution (or six drops in the case of the methyl red) were added to the solution in *B*

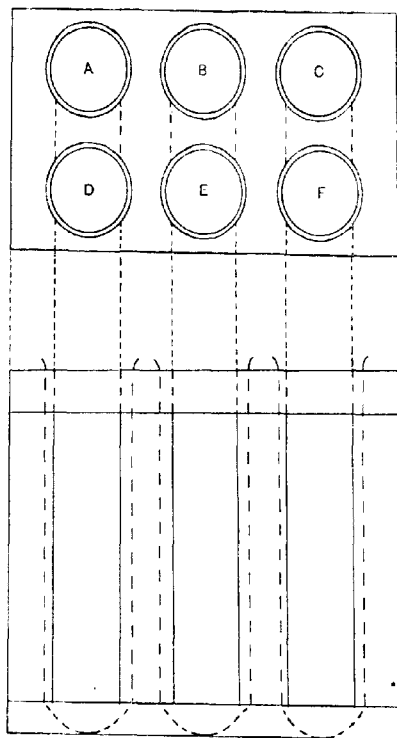


Fig. 2.

and the colour compared with those of *D* and *F*. By this arrangement the colour comparison could be fairly accurately carried out without any difficulty and with very little disturbing effect due to turbidity or natural colour. It will be noticed that in each of the three pairs of tubes the light must pass through the same total amount of liquid, of indicator, of turbidity and of natural colour, any disturbing effects due to

the last two factors being compensated for. The tubes *A*, *B*, *C* and *E* were closed with rubber caps similar to those on *D* and *F* to prevent any light from entering from above; if this occurred such light would be scattered by the particles in suspension and would be likely to affect the tint of an indicator showing dichromatism. A white paper or cardboard screen pinned to the front of the rack and just low enough to cut off the menisci from view is also desirable and has a sensible effect on the accuracy with which the colours can be matched or compared. With turbid liquids and especially indicators showing dichromatism such an arrangement possibly has an advantage over the Walpole tintometer and other types in which the light enters at the bottom and passes up the length of two superimposed tubes, in that errors due to dichromatism are very much reduced when the solutions are viewed in thin layers. There is an advantage also in using three pairs of tubes rather than two because the comparison tubes *D* and *F* can be so chosen that the colour of the solution to be tested (in *B*) is intermediate between them. With a little practice it is easy to judge whether the colour in *B* is exactly midway between that of *D* and *F* or whether it is more nearly that of *F* or *D*, so that, by a kind of visual interpolation, one can estimate the  $-\log [\text{H}^+]$  of the solution in *B* to 0.05 with a fair degree of accuracy.

As the approximate  $-\log [\text{H}^+]$  of the solution to be tested is generally not known preliminary tests were always made in test tubes of portions of the solutions with different indicators so as to find out the correct indicator to use. It was then merely a matter of finding a pair of standards such that one has a darker tint and one a lighter than the solution to be tested when all three are viewed side by side in the colorimeter. In all cases where two indicators overlap measurements were made with both indicators separately. There was generally good agreement except in those cases (of which there were few) where the turbidity or natural colour was so great that only approximate measurements of  $-\log [\text{H}^+]$  could be made. In such cases however the measurements were also made by the dilution method; that is the solution to be tested was diluted with conductivity water five or ten times and the  $-\log [\text{H}^+]$  measured in the usual way against standard solutions which had been themselves diluted to the same extent\*.

\* It can be shown theoretically as well as demonstrated that at the concentrations commonly employed in colorimetric work diluting ten times has a negligible effect on the  $-\log [\text{H}^+]$  of the solution. The present writer could detect no measurable effect on the colour of an indicator by diluting the standard solution ten times before adding the indicator.

The soil samples were taken with the usual precautions from the Rothamsted Experimental Grass Plots on the same day in August 1919; they were air dried, passed through the 3 mm. sieve (round holes) and stored in bottles.

Two courses were then open;  $-\log[H^+]$  measurements could be made on the filtered extracts or on the centrifuged extracts. The former were almost perfectly clear liquids, while the centrifuged extracts were always more or less turbid. Little is definitely known as to the effect the filter may have on the  $[H^+]$  of a solution passed through it, although some effect of adsorptive or other factors might be expected. Further it is uncertain whether turbidity has any effect on the  $-\log[H^+]$  of an extract other than that compensated for in the type of colorimeter used. It has been assumed generally that no such effects occur and the assumption has been supported in the case of culture media by the careful electrometric and colorimetric comparisons of Clark and Lubs and in the case of soils by the work of Gillespie and Hurst\*.

In the case of extracts filtered through paper or Pasteur-Chamberland thimbles it was found that as filtration proceeded the acidity of the filtrate increased so that the first portions of the filtrate always showed a larger  $-\log[H^+]$  than the later portions collected; while all gave different values from the centrifuged extracts. In consequence of this determinations were always made on the centrifuged extracts and in the preliminary work the procedure was as follows: 50 gms. soil (3 mm. sample) were mixed with 100 c.c. conductivity water in a stoppered bottle and shaken in an end-over-end shaker for an hour. They were allowed to stand a few minutes and the supernatant liquid decanted off and centrifuged for ten minutes at a speed of 3000 revs. per minute. Determinations were then made on the centrifugate as described above.

The results of preliminary experiments seemed to indicate little difficulty and perfectly definite values for  $-\log[H^+]$  were obtained, thus in a general way apparently confirming the results of other workers.

#### DETERMINATION OF LIME REQUIREMENTS COLORIMETRICALLY.

It was decided to extend the work so as to measure lime requirements as well as reaction alone. This could be done by adding varying amounts of calcium oxide to a series of mixtures of soil with twice its weight of water, shaking for an hour and measuring the  $-\log[H^+]$  after centrifuging. By plotting the values and interpolating at  $-\log[H^+] = 7.07$  the amount of lime required to bring the reaction

\* See however later, p. 64.



Table III, A. Samples of considerable Lime Requirements.

Plot No. →	9	9	10	11-1	11-2	4-2	18
c.c. N/5 Ba(OH) <sub>2</sub> added	(limed)	(unlimed)	(limed)	(limed)	(limed)	(limed)	
0	5.70 -	5.10	5.80 +	5.60 +	5.60 +	5.20 -	5.40 +
5	—	—	—	—	—	5.80 +	—
10	6.30	5.45	6.75	6.10	6.30 +	6.80 -	6.70 +
15	6.50 -	—	7.40 -	6.70 -	6.80	7.60 +	7.40
20	6.70 -	6.00 -	8.40	7.00 -	7.20	8.45	8.00 +
25	—	—	—	7.30	7.60 +	—	—
30	7.40	7.40	—	—	8.40	—	—
% CaO required to make -log [H <sup>+</sup> ]=7.07	0.295	0.317	0.129	0.244	0.207	0.129	0.141

Table III, B. Samples\* of low Lime Requirements.

Plot No. →	1	2	3	4-1	7	8	13	16	19	20
c.c. N/5 Ba(OH) <sub>2</sub> added										
0	6.00 -	6.70	6.70 -	6.60 -	6.60	6.60 -	5.50 +	6.70 -	5.80	6.10
0.5	—	7.00 -	7.10 -	—	7.00 +	—	—	7.10	—	—
1.0	—	7.40 +	—	7.20	7.50 -	7.20 +	—	—	—	—
1.5	—	7.80 -	—	7.60 -	—	7.60 -	—	—	7.10 -	7.10 -
2.0	—	—	—	7.90 -	—	7.90 +	—	—	—	—
3.0	7.30 -	—	—	—	—	—	—	—	—	—
4.0	7.70	—	—	—	—	—	—	—	—	—
5.0	8.20	—	—	—	—	—	7.10 -	—	—	—
% CaO required to make -log [H <sup>+</sup> ]=7.07	0.029	0.0067	0.0064	0.0084	0.0062	0.0084	0.056	0.0060	0.0224	0.0224

\* All these samples were from the limed plots.

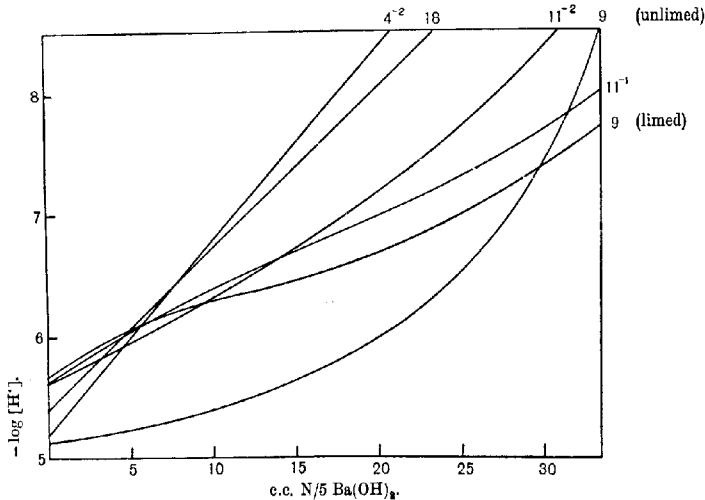


Fig. 3.

of the soil to this value could be calculated. Lime itself could not very well be used in practice. Saturated lime water was too dilute except where the lime requirement was extremely small. To weigh out numerous small quantities of pure  $\text{CaO}$  without contamination by moisture or atmospheric  $\text{CO}_2$  is too tedious an operation, while if  $\text{CaCO}_3$  were employed there is always the possibility that the decomposition would be slow or incomplete. Consequently until the validity of the colorimetric method could be established it was decided to use  $\text{Ba}(\text{OH})_2$  but to calculate the results in terms of lime.

It is evident of course that the  $-\log[\text{H}^+]$  values obtained colorimetrically do not represent the true values for the actual soil solution. They are the values obtained by shaking one part of soil with two parts of water for a definite time (one hour), always assuming that equilibrium has been attained during that time and that the method of determination is valid. The ratio soil/water = 1/2 was employed purely as a matter of convenience. But even if values for the  $-\log[\text{H}^+]$  so found are not strictly accurate they should be comparable from sample to sample and moreover the degree of dilution should not affect the value for the lime requirement obtained since once the mixture is neutral no reasonable amount of dilution should affect the reaction.

The values for  $-\log[\text{H}^+]$  and for percentage lime requirements (obtained by interpolation from the graphs) of 17 of the Grass Plot samples are given in Table III, A and B; and some of the results in Table III, A are shown graphically in Fig. 3.

#### EFFECT OF FINENESS OF DIVISION ON MEASUREMENT OF $-\log[\text{H}^+]$ .

The possible influence of fineness of division on the lime requirement of soil seems to have been almost completely overlooked and only three papers on the subject appear to have been published. Brown and Johnson(5) found that with certain sandy Iowa soils the lime requirement as found by the Veitch method diminished on grinding and to such an extent that in some cases soils having a high lime requirement before grinding actually became alkaline afterwards. Cook(9), on the other hand, with New Jersey soils using the same method found that in every case the lime requirement increased with grinding, and recommended in consequence that soils should not be ground if used for determination of lime requirement by the Veitch method. Sharp and Hoagland(23), using six soils from four American states, showed that the  $-\log[\text{H}^+]$  measured electrometrically was unaltered by grinding except in one instance when the value increased from 6.40 to 7.15.

This anomalous result, like those of Brown and Johnson, can apparently only be explained by the supposition that the interior of the soil particles concerned was of a different chemical composition from the exterior partially weathered layers\*.

If fineness of division does affect the  $-\log [H']$  of a soil and consequently the lime requirement as determined colorimetrically then the values given in Tables III, A and B, will probably be inaccurate. Moreover the supposition that fineness of division is a factor to be reckoned with is supported by the considerable curvature shown by some of the curves in Fig. 3, *e.g.* the curves for Plots 9 (limed and unlimed), and 11-2. As indicated in Fig. 1†, when considerable buffer action is present, neutralisation curves show very little curvature. In the case of soil-water mixtures with large buffer action and over a small range of

Table IV.

c.c. N/5 Ba(OH) <sub>2</sub>	Plot 10 (limed)				Plot 9 (unlimed)			
	3 mm. samples		100 mesh sample		3 mm. samples		100 mesh samples	
	I	II	I		I	II	I	II
0	5.80 +	5.30 -	5.40 -		5.10	5.10	5.40 -	5.30 +
10	6.80 - ; 6.70 +	5.50	5.50		5.40 +	5.50 -	5.40 +	5.40
15	7.40 -	—	—		—	—	5.50 +	5.60 -
20	8.40 +	6.30 - ; 6.60 -	5.80 + ; 6.00	6.50 - ; 6.40 +	6.00 -	—	5.80 +	5.80 +
25	—	7.00	6.30 +	—	—	—	—	—
30	—	7.80 +	6.90	7.40 + ; 7.40 -	7.40	6.50 ; 6.60	6.50	—
35	—	—	7.50	—	—	—	—	—
40	—	—	8.00	—	—	—	—	—

$-\log [H']$  very slight, if any, curvature should be shown. This was shown to be the case in the recent work of Knight. Moreover although it was generally easy to get a series of experimental points lying on or near a continuous curve when the whole series was done at the same time, if the series was repeated under slightly different conditions as to duration, manner and violence of shaking, quite different values and curves were obtained. If the samples were ground so that the greater part passed through the 100 mesh to the inch sieve little difficulty was

\* In all these cases the soil was actually ground up, *e.g.* in a porcelain mortar, so that a considerable amount of attrition occurred. In this investigation (see also Part I, pp. 41, 42) a wooden pestle was used so as to avoid actual attrition as the effect desired was a mere separation of the larger soil crumbs into the ultimate soil particles without any breaking up of these latter. It is unlikely that any fresh unweathered surface was exposed under these conditions and the case is not strictly comparable with those of the American workers.

† See also Figs. 1 to 3 in No. I of this paper.

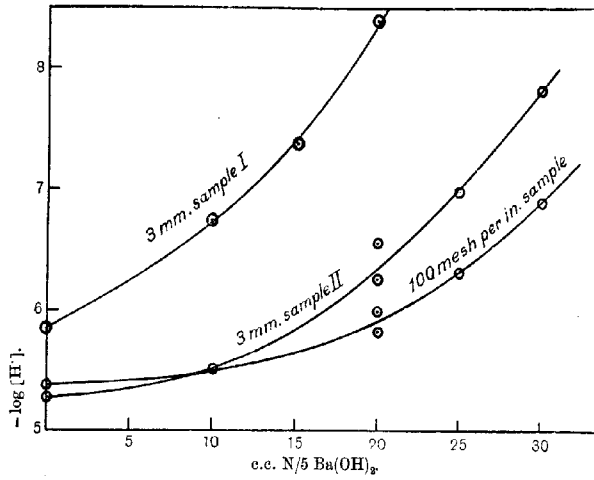


Fig. 4. Neutralisation curves for Plot 10 (limed end).

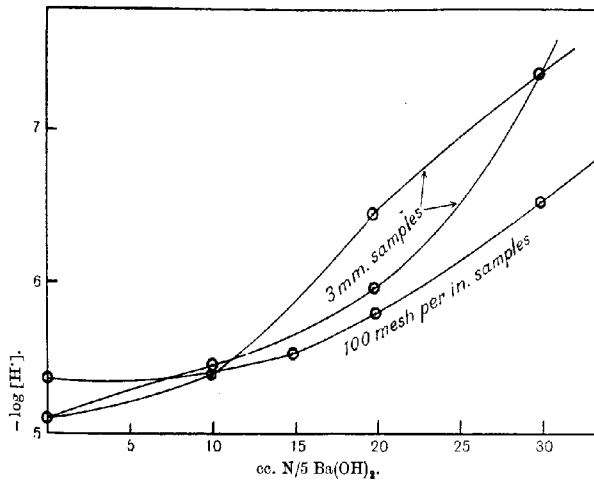


Fig. 5. Neutralisation curves for Plot 9 (unlimed end).

experienced in obtaining reproducible results. Table IV and Figs. 4 and 5 illustrate this point and bring out quite clearly the fact that fineness of division is apparently a serious factor to be reckoned with in acidity determinations.

Presumably the irregularities of the results from the 3 mm. samples are due to the alteration in effective absorbing surface resulting from the breaking up of the larger soil crumbs during the shaking. If this is so then the duration of the shaking should also have an effect on the apparent reaction and lime requirements of a soil, and no neutralisation curves should be accepted as correct unless they can be shown to be real equilibrium curves. Gillespie and his co-workers<sup>(14)</sup> used unground soil samples, the soil-water mixtures were shaken by hand fifty times and the extract centrifuged and its  $-\log [H^+]$  measured colorimetrically. In later papers the colorimetric results were compared with those obtained electrometrically, but apart from the satisfactory agreement found between the two lots of results there is nothing to show that equilibrium had really been attained. On the other hand, Gillespie did not attempt to measure lime requirements nor plot neutralisation curves, but only the actual  $-\log [H^+]$  of the soils investigated, and the disturbing effect of fineness of division and of time of shaking might be expected to be less on the latter than on the former. In this connection Sharp and Hoagland<sup>(23)</sup> in their electrometric measurements of soil reaction state with regard to the time taken for constant voltmeter readings to be obtained: "This occurs in the case of acid soils within a few minutes, but for soils approximately neutral a slightly longer time will be required. In the case of titrations prolonged shaking is required after each addition of the titrating solution, in order to obtain constant readings." They interpret this as being due to the "slow rate of solubility possessed by the acid constituents of the soil," but it can be equally well, if not more satisfactorily, explained as due to an increase in effective surface resulting from a breaking up of the crumbs during the shaking, especially as it is not at once evident why the rate of solubility of the acid constituents of the soil should be slower in the presence of an alkaline titrating liquid than in the presence of water alone. The results for Plot 4-2 (unlimed) given in Table V, columns *A*, *B* and *C*, and the corresponding curves in Fig. 6 were thought at first to throw some light on this matter: the 100 mesh sample gave consistently smaller values for  $-\log [H^+]$  than the 3 mm. sample shaken for the same time (one hour). On the other hand, the 3 mm. sample shaken for 30 hours gave very much smaller values for  $-\log [H^+]$  than either of the other samples.

Another explanation of this difference is however possible: it was noticed that the more finely ground a soil was or the longer the shaking the greater was the turbidity even after centrifuging, and it was thought that the variations observed might be due to some action of the suspended clay particles. To test this point a series of determinations was carried out on the same Plot 4-2 (unlimed), finely ground sample,

Table V. Plot 4-2 (unlimed).

c.c. N/5 Ba(OH) <sub>2</sub>	100 mesh sample shaken 1 hour (Curve B)*	3 mm. sample shaken 1 hour (Curve A)*	3 mm. sample shaken 30 hours (Curve C)*	100 mesh sample shaken 24 hours and flocculated with CaAc (Curve D)*
0	5.50	5.50 +	—	6.10
10	5.80	5.90 +	5.50	—
20	6.20	6.50 -	5.80 +	6.60
25	—	—	—	6.70 +
30	6.60	7.10	6.20 -	—
40	7.00	—	6.50	—

\* See Fig. 6.

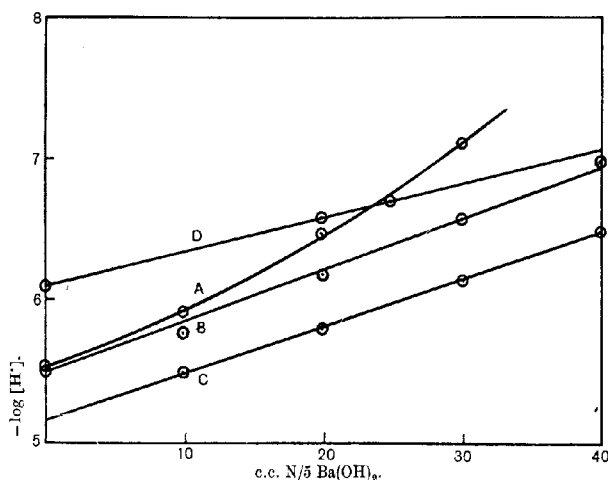


Fig. 6. Neutralisation curves for Plot 4-2 (unlimed end).

shaken for 24 hours. The soil-water mixtures were centrifuged for ten minutes, the extracts decanted from the sediment into a second lot of tubes, made N/100 with carefully neutralised calcium acetate solution and re-centrifuged for ten minutes. The calcium acetate causes a tolerably complete flocculation of the suspended particles thus eliminating the turbidity. The  $-\log [H^+]$  of these extracts was measured and the results are given in the last column of Table V and curve D of Fig. 6.

Clarifying with calcium acetate considerably decreased the acidity and also the apparent lime requirement from which it might be inferred that there is a turbidity effect due to the small amount of clay in suspension. No such turbidity effect was noticed in measuring the  $-\log [\text{H}^+]$  of bacterial culture media where the turbidity was due to agar. But agar is relatively non-reactive in comparison with the colloidal aluminosilicates that presumably make up the greater part of the turbidity of aqueous soil extracts, and it is possible that such compounds may exert some action on the indicator used such as selective absorption of acid or base or some disturbance of the tautomeric equilibrium determining the colour change. At the same time such effects are generally accompanied by difficulty in colour matching, and no such difficulty was observed.

The most probable explanation is to be sought in the nature of the substances present in the extract. If the acidity is due to sulphuric acid (produced, for example, by the hydrolysis of  $\text{Al}_2(\text{SO}_4)_3$ ) or acid phosphates capable of forming insoluble salts of lime, then the addition of Ca-acetate will result in the precipitation of an insoluble Ca-salt while a corresponding amount of acetic acid will be liberated. The  $[\text{H}^+]$  of the acetic acid produced would be diminished by the 'buffer action' of the Ca-acetate present. This would account satisfactorily for the observed differences in  $-\log [\text{H}^+]$  and is supported by the history of the soil used which had been manured for many years with sulphate of ammonia and superphosphate. Some other flocculant than Ca-acetate, preferably one of an insoluble or non-electrolytic character, is therefore desirable in eliminating turbidity. Possibly dialysed colloidal ferric hydroxide might answer the purpose as suggested by Gillespie.

Evidently a thorough investigation of the hydrogen-ion concentration of soils and aqueous soil extracts is very desirable. The present writer however has had to abandon the work at an early stage but it is hoped that the results so far obtained, although preliminary in character, may be found of interest.

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## THE PREPARATION AND FRACTIONATION OF HUMIC ACID.

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### INTRODUCTORY.

THE early authorities, Mulder, de Saussure, Berzelius and others, considered the whole of the precipitate obtained by adding acid to an alkaline extract of soil to be humic acid,—a single substance to which they assigned a definite formula. Hoppe-Seyler<sup>1</sup> and others found, however, that the precipitate was not homogeneous; a portion dissolved in alcohol, which he named hymatomelanic acid. It was from this that Schreiner and Shorey<sup>2</sup> isolated a number of organic acids, all of which were either colourless or only faintly coloured.

Sven Oden<sup>3</sup> obtained a colloid-free solution of ammonium humates by coagulating its free colloids and removing them by sedimentation and filtration. The total precipitate from this solution was extracted with alcohol leaving the insoluble humic acid, which he assumed to be homogeneous. Electrometric measurements on this residue demonstrated the existence of humate ions, to which he assigned the equivalent weight of 339, and a basicity of 3. Ehrenberg and Bahr<sup>4</sup> obtained their humic acid by filtering the alkaline extract through collodion; they also showed by electrometric measurements that the alkali humates behaved as salts of a true acid with a basicity between three and four.

The author attempted to determine the methoxy content of humic acid carefully prepared as described below, but was driven to the conclusion that the substance was a mixture and not homogeneous as previous workers have assumed. It was fractionated by pyridine, part being soluble and the rest insoluble<sup>5</sup>. A certain amount of decomposition probably took place in the pyridine solution. Both fractions were acidic in character and dissolved in dilute ammonia. It is quite possible that these acids are respectively tri- and tetra basic, in which case Ehrenberg and Bahr's results are readily explained.

<sup>1</sup> *Zeit. physiol. chem.* 1880, **13**, 62.

<sup>2</sup> *U.S. Dept. of Agric. Bur. of Soils, Bull.* **74** (1910).

<sup>3</sup> *Ber.* 1912, **45**, 651; *Internat. Mitt. für Bodenkunde*, 1916, **6**, 81; *Kolloid. Zeit.* 1914, **14**, 123.

<sup>4</sup> *Journ. für Landw.* 1913, **61**, 427.

<sup>5</sup> The solvent action of pyridine on some of the constituents of peat humus is recorded by Miklausz (*Zeit. für Moorkultur und Torferwertung*, 1908, **6**, 285).

## EXPERIMENTAL.

Air-dried soil, passing through a 3 mm. sieve, was introduced into Winchester bottles in 300 gm. lots and shaken with dilute hydrochloric acid, which was then washed out with distilled water. The soil was then shaken in an end-over-end shaker for  $2\frac{1}{2}$  hours, with a litre of 4 per cent. ammonia solution, containing 20 gms. ammonium chloride to coagulate the clay. The dark coloured solution was collected after the clay had settled, and the soil extracted twice with fresh ammonia solution; this removed practically all of the soluble melanoid organic matter.

The combined alkaline solutions were filtered first through a Berkefeld filter to remove the little suspended clay and a quantity of a black organic colloid, and then through a collodion filter<sup>1</sup>. The solution thus obtained was quite clear and deposited no colloid on standing.

On acidification the characteristic flocculent precipitate was formed, the supernatant liquid (Mulder's apocrenic acid) being of a wine-red colour. The precipitate was washed with acidulated distilled water till the washings were clear, after which distilled water alone was used till the solid peptised to a permanent suspension. By this time practically all the electrolytes were removed. The suspended matter was now filtered off on a collodion filter and washed several times with distilled water.

The precipitate was allowed to drain on the filter till drying contraction took place, whereby it broke up into large irregular lumps with clean sharp edges, easily and cleanly removed from the filter. These lumps were introduced into a round bottomed flask and boiled with 95 per cent. alcohol under a reflex condenser. The black solution contained a quantity of suspended matter which was filtered off on a No. 50 Whatman filter. Extraction with alcohol was continued till the extract became straw-coloured. The solid had contracted a great deal; it was ground up in alcohol, passed through a medium bolting silk, boiled with absolute alcohol, filtered through the filter used for the alcoholic extracts, and washed several times with boiling absolute alcohol till the filtrate had a very faint yellow colour. The dried solid was brown, peptised readily in water, dissolved immediately in dilute ammonia and did not become insoluble on heating. The alcoholic extract on evaporation gave a brown powder consisting of a melanoid body and

<sup>1</sup> The collodion filters were made by soaking filter papers in a 10 per cent. solution of dry pyroxylin in glacial acetic acid (M.P.  $15^{\circ}$  C.), till translucent. The excess of collodion was drained off, the filter being reversed to obtain an even coating, and the paper immersed in water. When all the acetic acid was removed by successive washings the filters were ready for use. These filters proved very satisfactory when used with a filter pump.

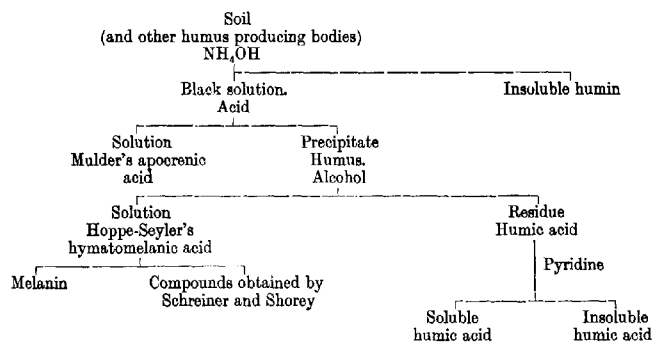
## 68 *The Preparation and Fractionation of Humic Acid*

the resin and other acids isolated by Schreiner and Shorey. About one-third dissolved in the author's experiments.

Its behaviour when treated with hydriodic acid in the determination of methoxy groups led the author to believe that the alcohol-insoluble humic acid was a mixture. Ether and the other solvents removed nothing: pyridine however dissolved about one-half. Humic acid was therefore extracted in a Soxhlet extractor with pyridine; a wine-red solution was obtained which gradually intensified in colour as the extract became concentrated, till finally it was black. When extraction was complete the pyridine was replaced by ether and extraction continued to remove pyridine from the residue. Much remained and was removed by washing with dilute hydrochloric acid. The acid was then washed out with water followed by alcohol. This pyridine-insoluble portion remained as a brown-black, easily pulverised body, which was immediately soluble in ammonia.

The pyridine-soluble portion was recovered from the extract by distilling off the pyridine to a small volume and neutralising with hydrochloric acid. The acid solution however had a deep red colour, which leads to the conclusion that a certain amount of decomposition took place in the pyridine extract, possibly due to oxidation at the high temperature.

Humus may therefore be fractionated according to the following scheme:



The above procedure has been repeated with rotted straw and with sugar humus, and in both cases the same fractions were obtained. The residue after pyridine extraction of sugar humus was however only slowly soluble in ammonia, probably having been converted into humin.

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## THE FORMATION OF HUMUS<sup>1</sup>.

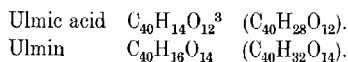
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### PREVIOUS WORK.

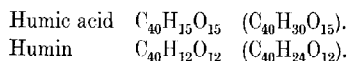
It was early noticed that soil on treatment with a solution of an alkali yielded a dark coloured solution which gave a black flocculent precipitate on acidification. Decomposing vegetable matter behaved in a similar way.

Since this substance was soluble in alkalies and insoluble in acids it was regarded as an acid. Mulder<sup>2</sup> considered that the brown substance formed on moors was identical with that obtained from rotting wood, diseased plants and similar sources. The soluble portion he named ulmic acid and the insoluble ulmin. To these he assigned the formulae



Ulmic bodies he considered the first products of decomposition of plant residues under aerobic conditions. Oxygen was absorbed, carbon dioxide evolved and the elements of water eliminated. The final product was richer in carbon and poorer in hydrogen and oxygen than the plant residues from which it was obtained.

The black bodies obtained from bogs and from soil under anaerobic conditions he named humic acid and humin. To these he assigned the formulae



<sup>1</sup> The terms "humus," "humic acid" and "humin" are used in this paper with the following meanings:

"Humus." The total precipitate obtained on the acidification of the alkaline solution from soil, etc.

"Humic acid." That portion of humus insoluble in boiling alcohol.

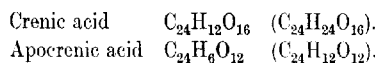
"Humin." The part insoluble in ammonia. There is also an insoluble modification of humic acid which may be identical with humin.

<sup>2</sup> *Chemie der Ackerkaune*, Bd. 1, 308 (1863 Edit.).

<sup>3</sup> In Mulder's day the equivalents of C and O were half of those now accepted, C=6, O=8. Modern formulae are given in brackets after Mulder's formulae.

The formation of humic bodies, Mulder considered was the second stage in the decomposition of plant matter.

The third stage in the decomposition of organic matter in the soil he considered to be the formation of apocrenic and crenic acids, already obtained by Berzelius from spring water. Crenic acid (Quellsäure) was colourless and on exposure to the air was converted into apocrenic acid (Quellsatzsäure) by oxidation. These acids were not precipitated with humic acid. Apocrenic acid could be obtained as an insoluble salt by adding a copper salt to the liquid left after the precipitation of humic acid. Crenic acid he obtained from apocrenic acid by reduction with zinc and hydrochloric acid. To these compounds he assigned the formulae



The humic bodies derived from sugars by the action of mineral acids were considered by Mulder to be closely related to soil humus, though not identical. The nitrogen in natural humus was apparently in the form of ammonium or related compounds, since most of it was liberated as ammonia on boiling with potassium hydroxide.

Conrad<sup>1</sup> and Guthzeit controverted the accepted theory that humus was formed from cane sugar and other disaccharides as such. They showed that inversion took place in every case and that laevulose gave more humus than dextrose. Laevulinic and formic acids were simultaneously produced and in greater quantities with increasing concentration of reacting acid.

It was still widely believed that this humus was identical with that from soil. Robertson, Irvine and Dobson<sup>2</sup> prepared the natural humus from heather peat, separating the precipitate by filtering through filter paper on a Buchner funnel. There was a difference between humus prepared through the ammonium and potassium compounds. The artificial compound was prepared from cane sugar and hydrochloric acid, the concentration of the acid being adjusted so as to produce a substance which approximated to the natural in appearance and solubility. Very little of this artificial compound was soluble in alcohol. Analyses and methoxy determinations showed that these substances all differed.

Bottomley<sup>3</sup> found that the carbon content of the insoluble residue of the natural body rose after extraction with alcohol. The residue so

<sup>1</sup> Ber. 1885, **18**, 439; 1886, **19**, 2569 and 2845.

<sup>2</sup> Biochem. Journ. 1907, **2**, 458.

<sup>3</sup> Ibid. 1915, **9**, 260.

obtained closely approximated in composition to the artificial substance extracted with alcohol. He assumed that neither the natural nor the artificial acids contained nitrogen. Robertson Irvine and Dobson, however, showed that the artificial acid prepared through the ammonium salt contained a small quantity of nitrogen, apparently combined.

Bottomley was struck by the changes in colour in the acid sugar solution, which changes increased in rate with the strength of acid. The rate of change was high with strong acids such as hydrochloric acid and low with weaker acids such as oxalic acid. Also, strong acids produced more insoluble humin.

#### THE REACTION YIELDING ARTIFICIAL HUMUS FROM SUGAR.

##### 1. *Mineral Acids.*

Kiermayer<sup>1</sup> obtained  $\omega$ -hydroxymethyl-furfural by heating a 30 per cent. solution of cane sugar with 0.3 per cent. oxalic acid. Fenton<sup>2</sup>, Erdmann<sup>3</sup> and others have noticed that hydroxymethyl-furfural gradually darkens in an open vessel and eventually changes to a humus-like mass. These observations suggest that the sugar, after inversion, breaks down to a hydroxymethyl-furfural which then condenses to form the artificial humus.

The writer's experiments confirm this view. In the first place it was shown that Fenton's observations are correct. A small quantity of hydroxymethyl-furfural exposed to the light and air in a covered crystallising dish changed in the course of six months from a yellow liquid to a black viscous mass, part of which dissolved in water to a yellow solution, the rest forming a dark brown residue.

The solution was shown to contain unchanged hydroxymethyl-furfural. The residue was washed with water and treated with a 4 per cent. solution of ammonia. Most of it immediately dissolved, the rest dissolved more slowly, giving a black solution which on acidifying yielded a brown precipitate, similar to many humic acid precipitates.

This change takes place very slowly at laboratory temperatures. On boiling an aqueous solution, or a 12 per cent. hydrochloric acid solution of hydroxymethyl-furfural, humus was more rapidly obtained. Furfural was also detected in the distillate by the rose colour with aniline acetate and the greenish-black precipitate with phloroglucinol.

<sup>1</sup> *Chem. Ztg.* 1895, **19**, 1003.

<sup>2</sup> *J. C. S.* 1909, **95**, 1338, and private information from Dr Fenton.

<sup>3</sup> *Ber.* 1910, **43**, 2391.

It was further shown that hydroxymethyl-furfural was formed in quantities when cane sugar was boiled with 3 per cent. hydrochloric acid<sup>1</sup>. The solution slowly changed to yellow then to red, red-brown, brown and finally black; then followed a deposition of humus, most of which was soluble in 4 per cent. ammonia. Samples of the solution were taken at various intervals, neutralised rapidly with powdered calcium carbonate, filtered, the residue washed twice with definite volumes of water, filtrate and washings being made up to 100 c.c. This was then extracted three times while still warm with its own volume of ether, purified by distillation over sodium. The extract was then dried over calcium chloride and the ether distilled off. The residue was weighed; it consisted of a yellow oily liquid which increased in quantity till precipitation of humus began. While humus was being formed it remained constant in quantity and dropped suddenly to a small amount when humus formation ceased.

Stage of reaction	Weight of residue gms.	Weight of phenylhydrazone <sup>2</sup> gms.
Brown ... ..	0.046	—
Opaque, brown black ... ..	0.255	0.315
Humus forming rapidly ... ..	0.393	0.316
Humus formation very slow ... ..	0.037	—
Humus formation nearly ceased	0.035	—

The smaller quantities of residue gave positive results for all the colour tests for hydroxymethyl-furfural. The larger quantities were converted into the phenylhydrazone by dissolving in water and treating with an aqueous solution of phenylhydrazine. The phenylhydrazone separated in the form of bright yellow plates. These were weighed and then recrystallised from dilute alcohol. At the same time the phenylhydrazone of the hydroxymethyl-furfural obtained by the method of Kiermayer was prepared. Melting points of each compound and of a mixture of the two were determined.

M.P. of phenylhydrazone from extracts ... ..	136.2° C.
M.P. of hydroxymethyl-furfural phenylhydrazone	136.5° C.
M.P. of mixed phenylhydrazones ... ..	137.0° C.

The residue from the ether extract was therefore largely hydroxymethyl-furfural.

<sup>1</sup> Dilute acid was used so as to make the colour changes slow.

<sup>2</sup> A solution of phenylhydrazine acetate gave a red oil or a sticky red paste with hydroxymethyl-furfural. This was soluble in alcohol but did not crystallise. An aqueous solution added carefully till phenylhydrazine was in slight excess gave beautiful golden plates. These were partially soluble in water and therefore the weights of the phenylhydrazone are not quantitative.

While the humus formation is taking place there is a copious evolution of furfural, which, however, like the laevulinic acid found by Conrad and Guthzeit<sup>1</sup>, is formed in the decomposition of hydroxymethyl-furfural.

## 2. *Amino Acids: Maillard's reaction.*

Maillard<sup>2</sup> found that a concentrated solution of a mono-saccharide and an amino-acid or polypeptide gave on heating a black precipitate similar to soil humus in its appearance, properties and percentage composition. He concluded that a similar change occurred in the soil, the protein of the plant and animal residues decomposing to polypeptides and amino acids which reacted with the carbohydrates of the residue to form soil humus.

In repeating Maillard's experiments a weaker solution of glucose and glycine was used; the mixture was kept boiling slowly in a glycerin bath at 110° C. The colour changes resembled those with hydrochloric acid and sucrose, but took place very much more slowly. When the solution became black a portion was neutralised with calcium carbonate and extracted with ether as before. Again a yellow liquid was obtained which gave a phenylhydrazone melting at 137° C. Further proof that the substance was hydroxymethyl-furfural was afforded by colour tests. Furfural was again detected in the escaping steam. These experiments show that humus is formed as stated by Maillard but that hydroxymethyl-furfural is formed as an intermediate product.

## THE OCCURRENCE OF FURFURAL AMONG THE REACTION PRODUCTS.

Roxas<sup>3</sup> observed the evolution of furfural on heating various amino acids and sugars with hydrochloric acid and suggested it as the intermediate compound in the formation of humus, the aldehyde groups condensing with the amino acids to form a ring compound.

Gortner<sup>4</sup> observed a black mass resembling humus on heating furfural with concentrated hydrochloric acid and argued that acids acting on carbohydrates produce furfural which then polymerises to form humus.

Maillard used a pentose but in all the other work hexoses and "hexosans" were used as the carbohydrate. Now the primary product of an acid on a hexose or a "hexosan" is not furfural but hydroxy-

<sup>1</sup> *Ber.* 1885, **18**, 443.

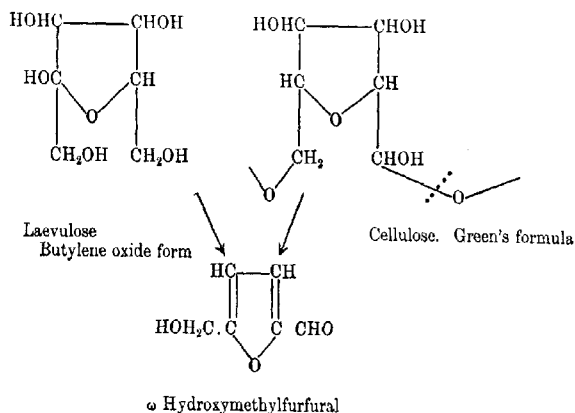
<sup>2</sup> *Compt. Rend.* 1912, **154**, 66; 1912, **155**, 1554; 1913, **156**, 1159.

<sup>3</sup> *J. Biol. Chem.* 1916, **27**, 71.

<sup>4</sup> *Ibid.* 1916, **26**, 177.



methyl-furfural. The ketohexoses and "hexosans" having a ketose structure such as inulin and cellulose<sup>1</sup> produce large quantities of hydroxymethyl-furfural or its substitution products.



The aldoses and "hexosans" such as starch produce smaller amounts of hydroxymethyl-furfural, and also smaller amounts of humus than the ketoses.

The amount of humus formed varies with the amount of hydroxymethyl-furfural.

It is thus unnecessary to postulate the formation of furfural to account for the production of humus. A solution of hydroxymethyl-furfural yields furfural and humus when boiled with 12 per cent. hydrochloric acid. It is therefore very probable that the furfural produced from hexoses and "hexosans" results from the decomposition of hydroxymethyl-furfural. Owing to the difficulty of purifying any quantity of hydroxymethyl-furfural without polymerisation, this problem could not be further investigated.

#### THE FORMATION OF NATURAL HUMUS.

Since the greater part of the plant residues in the soil are initially of a carbohydrate nature, it seemed probable that the formation of humus by bacterial and fungal action follows a similar path. Accordingly various sources of humus were examined.

<sup>1</sup> Green, *J. C. S.* 1906, 89, 811.

1. *Rotted straw.*

A quantity of straw to which had been added an amount of ammonium sulphate equivalent to its nitrogen content, was allowed to rot. It yielded large quantities of humus and closely resembled a well rotted stable manure. 500 gm. of this was air-dried, then mixed with plaster of Paris to absorb moisture, introduced into a Soxhlet extractor and extracted with redistilled ether which had been kept over sodium. The extract was bright green in colour; on distilling off the ether there remained a thick dark green oily liquid with a peculiar "strawy" smell, also possessed by the distilled ether.

A small quantity of distilled water was introduced into the flask which was shaken violently. The oily liquid was thus finely divided and finally adhered to the walls of the flask. The aqueous solution had a faint greenish yellow tinge and was practically odourless; it gave the tests for hydroxymethyl-furfural as shown below.

Reagent	Reaction with hydroxymethyl-furfural $C_6H_6O_3$	Reaction with aqueous solution	
Phloroglucinol in 12% HCl	Red solution and red precipitate	Slight red precipitate and solution	+
Resorcinol in 12% HCl	Red solution and red precipitate soluble in ethyl acetate on neutralisation	Red colour only. Absorption spectrum same as that of $C_6H_6O_3$	+
$\beta$ -naphthylamine in acetic acid (1:4)	Vivid yellow colour (characteristic)	Vivid yellow	+
Aniline acetate	Orange red colour (not sensitive)	Very faint colour	±
Alcoholic solution + thymol and few drops conc. $H_2SO_4$	Peculiar rose violet colour. On dilution with alcohol a delicate violet (sensitive)	All colour changes	+
$\beta$ -naphthol in conc. $H_2SO_4$	Purple (sensitive)	Purple	+

As would be expected from its unstable character a very small quantity of hydroxymethyl-furfural was obtained. As soon as produced this substance would polymerise to humus, very probably linking up in the chain any labile nitrogen compound.

2. *Humus in soil.*

Two samples of soil, one from Barn field dunged plot and one from a well-manured cucumber greenhouse soil, were extracted as described for straw. Both extracts were brown with a bright green fluorescence. When extraction was completed the soil was replaced by fresh soil; 1500 gms. of soil were thus extracted with the same lot of ether. The ether extract was then concentrated to a small volume and extracted

with successive small amounts of distilled water in a separating funnel. The aqueous solution again gave positive indications of hydroxymethyl-furfural. The residues from the extracts were brown pasty masses also possessing the "strawy" smell.

Reagent	Reaction with Barn field extract		Reaction with cucumber soil extract	
Resorcinol in 12% HCl	Red colouration	+	Red colouration	+
$\beta$ -naphthylamine in acetic acid (1:4)	Yellow colour	+	Yellow colour	+
Thymol + $H_2SO_4$	Rose violet; violet on dilution		Rose violet; violet on dilution	
$\beta$ -naphthol in $H_2SO_4$	Green on surface; purple + below		Green on surface; purple + below	

*Decomposition of Cellulose by Spirochaeta cytophaga.*

A large number of *Spirochaeta cytophaga* (Clayton and Hutchinson) were found growing on the rotted straw. In order to ascertain whether these may have produced hydroxymethyl-furfural from cellulose a series of flasks was put up containing filter paper and Kellermann's nutrient solution. After sterilisation at 120° C. for 20 minutes, inoculations were made with a subculture of organisms from the rotted straw. Growth was allowed to proceed for several months till a pulp covered with the characteristic yellow pigment was formed. The liquid was then filtered off at the pump, evaporated to a small bulk in vacuo at 25° C., filtered to remove the coagulated mucus and extracted with purified ether. Only faint indications of hydroxymethyl-furfural were obtained.

The experiment was repeated with larger quantities, but the unconcentrated filtrate was extracted with ether. The ether after concentration to a small amount had a faint green tinge. No indications of hydroxymethyl-furfural were found.

The experiment was repeated in a different way. A mixture of 200 gms. of Whatman filter paper ground up with 2000 gms. white sand, was run into a large aspirator bottle, moistened with Kellermann's solution and inoculated with an aqueous extract from the rotted straw. Air was drawn through at a slow rate for two hours a day. After a fortnight the sand on the walls blackened. The bottle was shaken to mix the contents thoroughly and the action allowed to proceed. Daily shaking was done before starting the stream of air. After a week the mixture was dried in a current of air at 60° C. Very little colour change had taken place throughout the mixture. This was, however, extracted as before, 1500 gms. being taken; no hydroxymethyl-furfural was found. The product was a light brown pasty mass soluble in ether with a faint

green fluorescence. The aqueous solution from this gave with thymol and sulphuric acid a colour somewhat like that from hydroxymethyl-furfural, but not identical;  $\beta$ -naphthol and sulphuric acid gave an emerald green solution with a vivid green fluorescence. There is therefore no evidence as yet of the formation of hydroxymethyl-furfural by *Spirochaeta cytophaga*.

#### CONCLUSIONS.

Evidence is adduced that the formation of humus both in the laboratory and in the soil proceeds in two stages:

Carbohydrates react with acids (whether mineral or amino acids) to produce hydroxymethyl-furfural;

Hydroxymethyl-furfural condenses to form humus.

In addition, in the laboratory, there is produced some furfural and laevulinic acid.

No evidence of the formation of hydroxymethyl-furfural during the decomposition of cellulose by *Spirochaeta cytophaga* could be obtained.

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## WET AND DRY FEEDING OF CONCENTRATES TO DAIRY COWS.

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### INTRODUCTION.

IN the winter feeding of cows in milk there is a diversity of opinion among dairy farmers as to the extent to which it is desirable to "prepare" the food. Some believe in expending no labour which can be avoided in the preparation of the food; the roots are fed whole, the hay and straw are used in their natural state, and the cakes and meals are given dry. Others slice or pulp the roots, and the concentrates are fed as wet mashes. In some cases the cakes and meals in a sloppy condition are mixed with chaffed fodder and sliced roots, and distillery by-products such as draff are added to impart flavour as well as to furnish nutritive material. The mixture (chopp), which is sometimes further moistened with water, is left over-night to allow of a certain amount of fermentation, which is carried on at the expense principally of the digestible substances, to set in, the object being to make the food more appetising.

For the same reason steaming is sometimes resorted to. Steaming and cooking lower the digestibility of the proteins<sup>1</sup> and render inactive the vitamine element<sup>2</sup> of the food. Nevertheless to pigs foods such as potatoes are more suitable for feeding in the cooked compared with the raw state<sup>3</sup>. The softening of the food which results would be an advantage to ruminants when the material is so hard that it might otherwise escape mastication and digestion, but in that case it would perhaps be better to deal with it by grinding.

Cooking effectively destroys all forms of bacterial and fungoid growth, a result which would prove of use in the case of damaged and partly decomposed foods, but normal foods are rarely if ever benefited on this

<sup>1</sup> Warrington, R. *Chemistry of the Farm*. Vinton & Co., London, 1909, p. 150.

<sup>2</sup> *Report upon present state of knowledge concerning accessory food factors (Vitamines)*. Medical Research Committee, H.M.S. Printing Office, 1919, chap. IV, pp. 49-67.

<sup>3</sup> Kellner, O. *Scientific feeding of Animals*. Duckworth & Co., 1909, p. 179.

account<sup>1</sup>. Steeping in cold or tepid water softens the food without diminishing its digestibility, unless the food contains nutritive substances soluble in water and the steep water is thrown away, then it would be lowered. Poisonous compounds soluble in water can be removed from foods by this means.

A method followed on many dairy farms in the south and south-western counties of Scotland, is to mix the meals over night with hot or boiling water. The following morning sufficient hot water is added to raise the temperature to body heat and the whole is fed in a sloppy condition. It is claimed that the cows respond in milk better to the feeding of the meals "wet" compared with that from the same meals fed "dry."

Preparation or unnecessary handling of food increases the cost of feeding and consequently that of milk production, and unless an equivalent return is obtained the labour and expenditure involved could not be justified. For this reason it was decided to investigate the merits of the practice of feeding concentrates to dairy cows, (1) dry, and (2) scalded and given in a sloppy condition at body temperature; the effects on the yield of milk and on the health of the animal were to be taken as the principal bases for comparison.

It was anticipated that the difference likely to be obtained as a result of the feeding by the two methods would not be large and might come within the limits of the experimental error. As not more than ten cows were likely to be available at one time, at the College experimental station at Kilmarnock, grouping the cows into two balanced lots according to: period of lactation, milk yield, age of cow, date of service, etc., then feeding one lot on the dry and the other on the wet food, and taking the difference in the milk yield as being due to the difference in the feeding, was a method which could not be adopted. Unless a large number of cows were employed the experimental error would in all probability be greater than the difference it was sought to measure. The nature and the extent of the experimental errors, which are associated with investigations on milk production, were brought home to the author when carrying out an investigation on the summer feeding of cows on pasture, in which fifteen lots totalling 224 cows were employed<sup>2</sup>.

<sup>1</sup> *Cyclopedia of American Agriculture*, Macmillan & Co., 3, 1903.

<sup>2</sup> Berry, R. A. "Feeding of dairy cows on pasture." *W. of Scot. Agr. Coll.*, Glasgow, 1916, *Bull.* 76.

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### PLAN OF EXPERIMENT.

Six animals calving within 35 days of each other were available in the autumn of 1913, and they were separated into two groups. The plan adopted for the feeding was to divide the experiment into four equal periods. In the first period the animals in group 1 received the concentrates wet and alternately dry and wet in the following periods, while the animals in group 2 were fed in the reverse order, that is starting with the dry meal in the first period. The object of this arrangement was to bring out the effect, if any, of an advancing lactation and of varying weather conditions on the two methods of feeding. The allowance of roots, fodder, etc. was the same throughout.

The morning and evening milk respectively of each cow were weighed daily and the milk fat determined in each. A daily record was kept of the rainfall, humidity and maximum and minimum temperatures of the outside air. Other circumstances which might influence the result such as indisposition, change of milkers, etc., were recorded. At the commencement, sufficient fodder, roots and concentrates to cover the whole experiment were laid aside.

#### *Daily ration for each cow.*

	lbs.
Swedes ... ..	30
Hay ... ..	7
Oat straw ... ..	10
Bean meal ... ..	3
Crushed oats ... ..	3
Decorticated cotton cake ...	3

This supplied in lbs.: dry matter 25·8; dig. protein 2·2; dig. oil 0·62.  
Dig. carbohydrates and fibre 12·6.

#### *Daily programme.*

- 5.0 a.m. Milking.
- 6.0 a.m. Concentrates  $4\frac{1}{2}$  lbs. fed wet or dry. Straw ad lib. about 5 lbs.
- 8.15 a.m. Turnips sliced 15 lbs.
- 10-10.30 a.m. Cows put out to drink.
- 11.0 a.m. Hay 7 lbs.
- 3.0 p.m. Turnips sliced 15 lbs.
- 4.0 p.m. Milking.
- 5.0 p.m. Concentrates  $4\frac{1}{2}$  lbs. fed wet or dry. Straw ad lib. about 5 lbs.

*Method of feeding concentrates.* 1. *Wet.* Equal weights of bean meal, crushed oats, and decorticated cotton cake were mixed and scalded at 3 p.m. at the rate of  $\frac{1}{2}$  gallon boiling water to 9 lbs. of mixed meals to

which was added 1 oz. of rock salt. In the following morning one half of the daily allowance was warmed to body temperature by adding  $\frac{1}{4}$  gallon of hot water and fed to the animal, and in the afternoon the remaining half of the meal was warmed and fed in the same way. The meal for each animal was weighed out daily into a numbered bucket.

2. *Dry*. The mixed meals were fed in their natural state, one half of the daily allowance ( $4\frac{1}{2}$  lbs.) being given in the morning and the remainder in the evening.

Nutted decorticated cotton cake was used for the dry feeding and the same ground to a meal for the wet feeding. The nutting and mealing of the cake was done locally.

A preliminary trial lasting three weeks preceded the actual experiment and in which one half of the meals were fed "dry" in the morning and the remainder "wet" in the evening.

#### EFFECT OF SHORT PERIODS OF FEEDING.

Each period was of five weeks' duration. The average daily yield of milk in lbs. obtained for the feeding of the meals wet and dry respectively was as follows:

	Wet	Dry	Wet	Dry
Group 1.	24.8	24.1	24.8	20.0
	Dry	Wet	Dry	Wet
Group 2.	22.1	23.5	24.1	29.9

A comparison of the first three periods shows that the wet meal produced an average daily increase amounting to about  $\frac{3}{4}$  lb. of milk per cow per day, over that produced by the dry meal. With the feeding in the reverse order (group 2) the result obtained also showed an advantage, though not so definite, in favour of the wet meal. In the last period the fall in the milk yield as a result of an advancing lactation, was less when the cows received the wet compared with the dry meal (group 1).

A similar result was obtained in a ten week experiment carried out in the spring of the same year, when eight animals calving within 41 days of each other were employed. The experiment was divided into two periods of five weeks each. The average figures obtained in lbs. of milk were:

	Wet	Dry
Group 1.	29.3	23.4
	Dry	Wet
Group 2.	29.0	25.4



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In Fig. 1 below, the average daily yield of milk in lbs. per week for the cows in each group, the weekly rainfall in inches, the mean weekly temperature (average daily readings of maximum and minimum thermometers) and the humidity of the atmosphere (the daily differences between the readings of the wet and dry bulb thermometers) are represented in the form of curves.

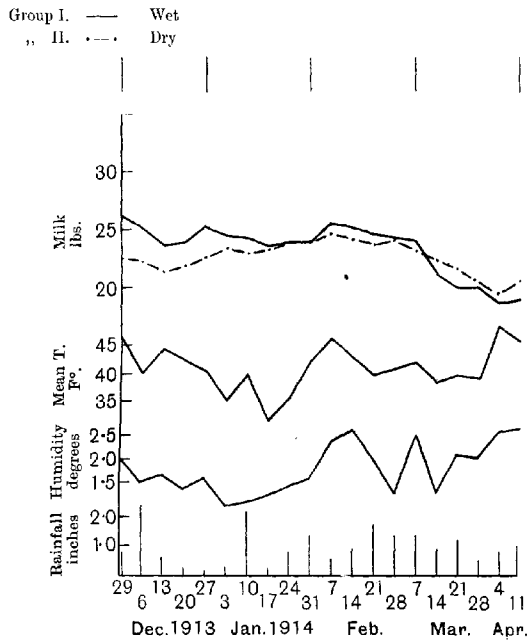


Fig. 1.

When examining the curves as well as the figures given (p. 81), it must be kept in mind that generally from about the fourth to the sixth week after calving, the curve for a normal milk yield would show a slow but continuous fall to the end of the lactation, and there would be a gradual rise in the percentage of milk fat. Further the effect of the feeding in one period may extend into that of the succeeding period. It is a well established fact that liberal supplies of suitable food stimulate the process of milk secretion, thus with a change from a limited to an ample supply of food, there is, as a rule, either a rise in the yield of milk,

or the yield may remain stationary, and the fall due to an advancing lactation temporarily arrested. Moreover, it is a matter of common experience that a change of food or of environment is generally reflected in the yield of milk. Then there is the question of the existence of galactagogues<sup>1</sup> and their effect on milk secretion, besides other factors which do not arise at this point, which go to make up the complicated and yet obscure process of milk formation.

A feature at once noticeable in the above graphs is the similarity in the form which the two milk curves take. Also there is the indication, more clearly shown in (curve) group I. that a change in the method of feeding irrespective of whether it is to the wet or to the dry meals affects the milk yield, a result already shown in the figures given. It is plain, however, that the irregular form taken by the two curves is not the result of the feeding only. Whether the variable air temperature has had any influence on the milk yield is not clear from the graphs but the point is worth keeping in view (see p. 95).

As a rule the cows did not take quite as readily to the change from the wet to the dry as from the dry to the wet feeding. If in the first day of the change, any meal was left untouched in the trough, it was consumed in the following day.

#### EFFECTS OF LONG PERIODS OF FEEDING.

A ten week instead of a five week period for the wet and dry feeding respectively was tried. It was thought that any effect produced would more likely be brought out by prolonging the periods. In other respects the details of the experiment were the same. Daily observations such as the health, appetite, etc. of the cows, the weather conditions, and any other circumstances which it was thought might influence the result, were recorded.

#### *Daily ration for each cow.*

	lbs.
Turnips ... ..	30
Hay ... ..	7
Oat straw ... ..	10
Bean meal ... ..	3½
Crushed oats ... ..	3½
Decorticated cotton cake ...	2

<sup>1</sup> McCandlish, A. C. "Possibilities of increasing milk and butter fat production by the administration of Drugs." *Jour. Dairy Sci.*, **1**, No. 6, 1918, 475-486. Gavin, W. "Quart. Jour. Exper. Physiol.", 1913. Hammond, J. and Hawk, J. C. "Studies in milk secretion." *This Journ.*, **8**, Pt. 2, 1917, 139-153. Hays, F. A. and Thomas, M. G. "Effects of drugs on milk and fat production." *Jour. Agr. Research (U.S.)*, **3**, 1920, pp. 123-130.

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This supplied in lbs.: dry matter 27·3; dig. protein 2·45; dig. oil 0·51; dig. carbohydrates and fibre, 13·37.

Ten animals calving within 45 days of each other were available. They were divided into two groups and the feeding of the concentrates was as follows:

	Group 1	Group 2
5 weeks	Dry	Wet
10 "	Wet	Dry
2 "	Dry	Wet

*Influence on the milk yield.* The result of the feeding on the milk yield is shown in the average daily yield in lbs. given below:

	Dry	Wet	Dry
Group 1.	29·0	26·2	24·0
	Wet	Dry	Wet
Group 2.	28·4	24·4	23·0

Owing to the periods not being of equal length the average yield of milk in one period is not comparable with that of another, but a comparison of the milk yield for group 1 can be made with that for group 2. This comparison shows that the advantage in favour of feeding the wet meal is equal to an increase of more than 1 lb. of milk per head per day over that produced by the dry meal. The advantage diminishes as the lactation advances.

The effect of the feeding on the milk yield is shown graphically in Fig. 2, p. 85, along with curves giving the mean temperature and the average weekly rainfall.

After a long period of feeding with the dry meal a change to the wet meal is marked by a perceptible rise in the yield of milk, whilst with a change from the wet to the dry the yield remained stationary for a time.

The milk yield curves are more regular than those given in Fig. 1, p. 82. The temperature is also not so variable.

Other effects besides that on the milk yield produced by the feeding, were observed during the progress of the experiment, and were brought out as a result of extending the length of the period of feeding. They are given below.

*Influence on the dung.* The dung from the wet fed animals was loose and there was a tendency on the part of the animals to scour, whilst from the dry fed lot the dung was harder and drier.

*Influence on the condition of the coat of the animals.* The coat of the wet fed lot retained its condition throughout the period while the animals

were receiving the wet food. After a change to the dry feeding the coat gradually got out of condition, the hair became dry and there was less gloss on it. With a change back to the wet feeding the coat regained its "bloom" again.

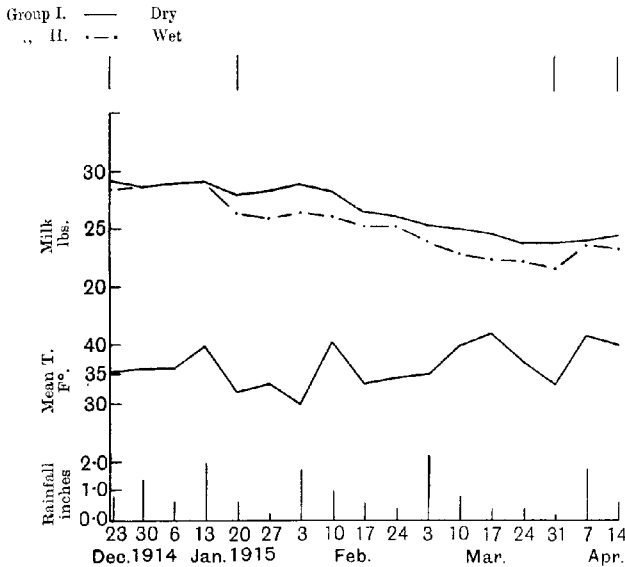


Fig. 2.

It was the opinion of Mr Young, who was in charge of this year's experiment, that the dry fed animals maintained their general health better than the wet fed lot. The feeding of sloppy food warm, if carried to extreme, deranges the digestive organs and renders the cows liable to chills<sup>1</sup>. But it can hardly be said that the feeding of warm sloppy food was carried to extreme in this case. However, continued looseness of the dung and a tendency to scour when spread over a long period, might eventually lower the condition of the animal and make it more susceptible to illness.

The warm water consumed daily in the sloppy meals amounted to one gallon. As the only difference between the two methods of feeding consisted in giving the concentrates warm and sloppy in one case and dry in the other it would appear that a difference in the amount of water

<sup>1</sup> *Cyclopedia of American Agriculture*. Article on Cow-keeping, 1, 1907. Green & Sons.

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consumed was probably the factor which affected the condition of the dung and of the coat of the animals.

*Water supply.* Drinking water was obtained from a trough in the yard, access to which was given along with the rest of the herd from 10 to 10.30 a.m. each day. In cold weather some of the cows would return to the byre without going to the trough. Considering the great importance of water in the animal dietary, especially in the case of cows, who, in addition to the supplies necessary for the ordinary physiological functions which it has to perform in the body, require considerable amounts for milk production, and in view of these results, the possibility of the cows not always taking an adequate supply of drinking water, suggested itself. It was decided to put the point to a test.

A long narrow metal trough was placed at the back of the stalls, resting against the wall, below the rack, but high enough so as not to interfere with the cows eating from the trough let into the floor. The cows were given access to the water for about one half hour daily by lifting a lid which was afterwards closed for the rest of the day. The trough was divided by a partition, each half was graduated so that the total water consumed per day by the wet and dry fed animals respectively could be measured.

Drinking from the trough commenced in the second week in March and was continued until the end of the experiment.

*Drinking water consumed.* As would be expected the animals receiving the dry meal drank more water than the lot getting the wet meal. The total water consumed was as follows:

	Gallons	Average per cow per day Gallons
Dry fed lot	1275	5.7
Wet    ,,	1065	4.6

The wet fed lot consumed an extra one gallon of water per day with the concentrates, but taking that into account the average total amount of drinking water consumed is slightly more for each of the dry fed animals. The latter animals also seemed keener for the water. Before the trough was put in, it was noticed that the wet fed animals took to the fodder better than the dry fed lot, although the amount of fodder taken daily was the same in each case.

*Influence of liberal water supply.* With accessibility to water made easier by the introduction of the trough into the byre, the differences in the appearance of the coat of the animals and in the texture of the dung which had previously characterised the result of the two methods of

feeding the concentrates, became much less pronounced. In the case of the condition of the coat of the animal the difference practically disappeared, whilst the dung from the dry fed animal became softer. The advantage in the milk yield in favour of the wet meal over that of the dry meal was maintained, showing that the increased water supply although it improved the condition of the coat of the dry fed animals, it did not appear to influence the respective milk yields. The improvement in the coat was similar to that produced by feeding the wet meal following a period of feeding with the dry meal.

When examining the milk yield curves of individual animals, temporary increases were noticed. It was found that these corresponded with the week following date of service and it occurred in the case of four cows. Fleischmann<sup>1</sup> states that the effect of bulling is to produce an immediate decrease in the yield of milk and fat, but almost directly afterwards the fat for a day or so is unusually high.

In the winter of 1915-16 some of the cows in the herd aborted, and owing to the war conditions then prevailing, there was a scarcity of, and a very limited selection of, foods available. In addition there was a great difficulty in procuring a suitable person to look after the experiment. As a result, further experimental work of this kind had to be postponed and it was not resumed again for several years.

#### WATER CONSUMPTION AND MILK PRODUCTION.

It was decided to repeat the wet and dry feeding test under the same conditions as that of the latter part of the 1915 experiment when a liberal water supply was available. Arrangements were also made to measure the amount of drinking water consumed daily by each cow and to determine the amount contained in, and taken in, the food.

Except for the components of the ration the other details of the experiment were the same as in previous years. The water was given in buckets and each animal was allowed to drink as much as it wanted and the quantity measured. The cows were allowed out for exercise daily.

#### *Daily ration for each cow.*

	lbs.
Swedes ... ..	30
Hay ... ..	7
Oat straw ... ..	10
Oats crushed ... ..	3
Fish meal ... ..	1
Miller's offal ... ..	1
Earth nut cake ... ..	4

<sup>1</sup> Fleischmann, W. *The Book of the Dairy*. Blackie & Sons, 1896, p. 40.

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This supplied in lbs.: dry matter, 27·4; digestible protein, 3·3; dig. carbohydrates and fibre, 12·3 and dig. oil, 0·6.

Four animals calving within 17 days of each other were available. The feeding of the concentrates was as follows:

4	weeks	on	the	wet,
7	..	..	dry,	
4	..	..	wet.	

The yield of milk in lbs., and the water consumed in gallons, also the mean weekly temperature and the rainfall are shown in Fig. 3 below. The mean temperature was less variable than that shown in Fig. 1, p. 82.

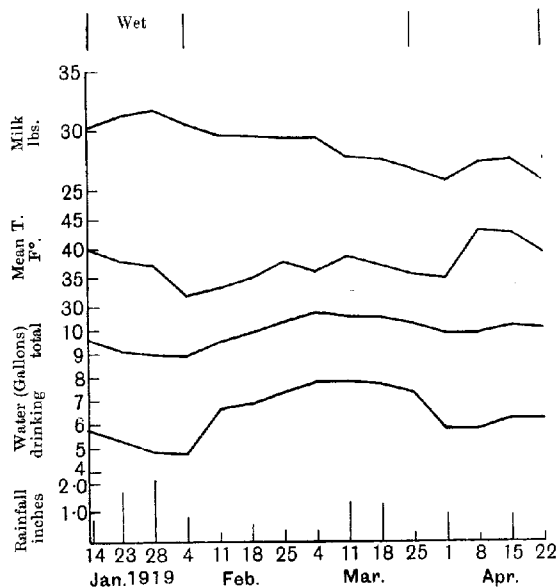


Fig. 3.

The result, in so far as the effect of the two methods of feeding on the milk yield was concerned, confirmed that for previous years. No difference however could be detected in the appearance of the coats of the animals as a result of feeding the food either wet or dry when a liberal water supply was available, but the dung from the animals receiving the wet meal was softer than that from the animals consuming the dry meal.

The laxative effect produced by the wet meal can hardly be attributed to a difference in the amount of water consumed as the total quantity taken in the case of the dry fed animals was slightly higher than that consumed by the wet fed lot. The inference is that the laxative action is due to the consumption of the concentrated food in a warm sloppy state. Whatever may be the action on the efficiency of the digestive changes, it is plain that the feeding of the sloppy food, as indicated by the increased milk yield, reacts favourably upon the process of milk secretion. A fact which would indicate increased rather than a diminished absorption of nutritive material as a result of adding boiling water to the meal and feeding warm. A ration producing a slight laxative effect is considered to be an advantage to dairy cows.

*Milk fat.* The milk fat was determined in both the morning and evening milk of each cow in each of the experiments. When the results are represented graphically, the percentage of fat was shown to rise and fall in a general way, in the opposite direction to that of the milk yield.

*Water requirements and milk production.* The intake of water for 1 lb. of milk produced, also the total daily supply received by each cow is given in Table I.

TABLE I.

Number of animal	Wet Meals			Dry Meal			Wet Meal			Average		
	Milk yield daily lbs.	Total water taken daily lbs.	Total water to 1 lb. milk produced	Milk yield daily lbs.	Total water taken daily lbs.	Total water to 1 lb. milk produced	Milk yield daily lbs.	Total water taken daily lbs.	Total water to 1 lb. milk produced	Milk yield daily lbs.	Total water taken daily lbs.	Total water to 1 lb. milk produced
4	33.1	100.9	3.0	28.6	105.8	3.7	24.1	108.4	4.5	28.6	105.0	3.7
19	29.1	85.7	2.9	27.0	99.9	3.7	26.5	92.7	3.5	27.5	92.8	3.4
28	26.5	76.8	2.9	24.5	100.4	4.1	21.9	87.6	4.0	24.3	88.3	3.7
35	35.8	103.8	2.9	34.1	112.5	3.3	33.4	110.2	3.3	34.4	108.8	3.2
average	31.1	91.8	2.9	28.5	104.6	3.7	26.5	99.7	3.8	28.7	98.7	3.5

To produce 1 lb. of milk a cow requires, on an average, about 3.5 lbs. of water including drinking water and water contained in the food, when fed in the winter months on a ration containing 30 lbs. turnips, and part of the concentrates given as wet mashes. The ratio varied with individual cows. It increased as the milk yield diminished.

The feeding also affected the ratio as the total intake of water for the dry fed animal was greater than when the animal was fed on the wet



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meal. McCandlish and Gaessler<sup>1</sup> found that 550 lbs. of water are required for 100 lbs. of milk produced in the summer.

The ratio would vary also according to the proportion of fodder in the ration. It is stated that for 1 lb. of dry matter taken in the food, 4 lbs. of water are consumed<sup>2</sup>. In the present experiment the ratio was 3.6 lbs. of water for 1 lb. of dry matter consumed.

As would be expected, the water requirements increased as the milk yield increased, and as the weather got warmer. The smaller animal, as it presents a relatively larger surface area for evaporation, consumed a relatively greater proportion of water than a larger animal.

The proportion of water contained in the milk to the total intake of water amounted in the present case on an average to about 26 per cent., and the remainder is made up of water secreted and lost by evaporation. About 62 per cent. of the total water taken was drinking water.

TABLE II.

Animal		Wet Meals		Dry Meal		Wet Meal		Average	
Number	Weight cwts.	Percentage of total water		Percentage of total water		Percentage of total water		Percentage of total water	
		drinking water	in milk	drinking water	in milk	drinking water	in milk	drinking water	in milk
4	10.5	60.1	28.8	74.6	25.0	62.8	21.1	65.8	25.0
19	12.0	53.2	32.3	69.2	24.0	56.7	23.2	59.7	26.5
28	12.25	48.1	30.2	69.8	21.6	54.3	19.2	57.4	23.7
35	10.0	61.3	30.5	73.6	26.8	63.7	29.3	66.2	28.9
Average		55.7	30.5	71.8	24.4	59.4	23.2	62.3	26.0

The average percentage of water in the foods used in these experiments was:

	%
Bean meal ... ..	14.1
Crushed oats ... ..	13.5
Paisley meal... ..	15.0
Miller's offal ... ..	13.2
Decorticated cotton cake ...	9.6
Earth nut cake ... ..	10.1
Bran ... ..	13.5
Fish meal ... ..	13.0
Hay ... ..	14.1
Oat straw ... ..	14.0
Swedes ... ..	90.0

<sup>1</sup> McCandlish, A. C. and Gaessler, W. G. "Water requirements for milk production." *Journ. of Dairy Science*, 2, No. 1, 1919, 8.

<sup>2</sup> *Cyclopedia of American Agriculture*, 3, 1908.

It was a practice in some of the milk selling farms in the west of Scotland to feed heavy root rations amounting to a hundredweight of roots per day. Dunstan<sup>1</sup> states that over 100 lbs. of roots are fed per head per day on some of the farms in Kent. A presumption is that drinking water is essential for dairy cows even when fed on a succulent diet<sup>2</sup>. Lauder and Fagan<sup>3</sup> found that cows receiving 112 lbs. of roots per head per day with wet mashes refused drinking water. In this experiment the total water consumed for moderate milking cows in winter amounted to about 10 gallons per day (Table I, p. 89). This quantity would be supplied by one hundredweight of roots.

#### DRINKING WATER AND WATER SUPPLIED IN SUCCULENT FOODS.

The effect of supplying the water requirements in the form of succulent food compared with a ration in which part of the water was taken as drinking water was tried.

*Plan of experiment.* In the first period the cows were given 112 lbs. of roots daily along with fodder and concentrates. In the second period the allowance of roots was reduced by one half while the fodder and the concentrates were adjusted so as to supply the same weight of proteins, fats and carbohydrates. The last period was the same as the first. The introduction of the heavy root ration was spread over about ten days. Eight cows calving within 51 days of each other were available.

#### *Daily ration for each cow.*

	lbs.
Swedes	112
Hay	5
Oat straw	12
Mixed concentrates	7

The mixed concentrates were made up in the following proportion: 2 lbs. each of bean meal, earth nut cake, oat hash, bran and 1 lb. fish meal. One half was fed dry and the remainder in a sloppy condition.

The ration supplied in lbs.: dry matter, 28.3; dig. protein, 2.8; dig. oil, 0.37; dig. carbohydrates and fibre, 15.2.

The yield of milk in lbs., the percentages of fat, and of solids-not-fat are shown in Fig. 4, p. 92, along with the consumption of water in gallons and the weather conditions.

<sup>1</sup> Dunstan, W. R. "Cost of feeding in milk production." *Trans. H. and Ag. Soc. of Scot.* Fifth series, **23**, 1911, 147.

<sup>2</sup> McCandlish and Gaessler, *ibid.*

<sup>3</sup> Lauder, A. and Fagan, T. M. "The effect of heavy root feeding on the composition and yield of milk." *Bull.* 26. Edin. and E. of Scot. Ag. Coll., 1912.

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The effect of the heavy root feeding was to convert a distinct tendency on the part of the milk yield to increase into a definite decrease which persisted for two weeks after the heavy root feeding had given place to one half the allowance of roots. The milk yield of the cows in the several weeks preceding the commencement of the heavy root feeding is not shown in the graph.

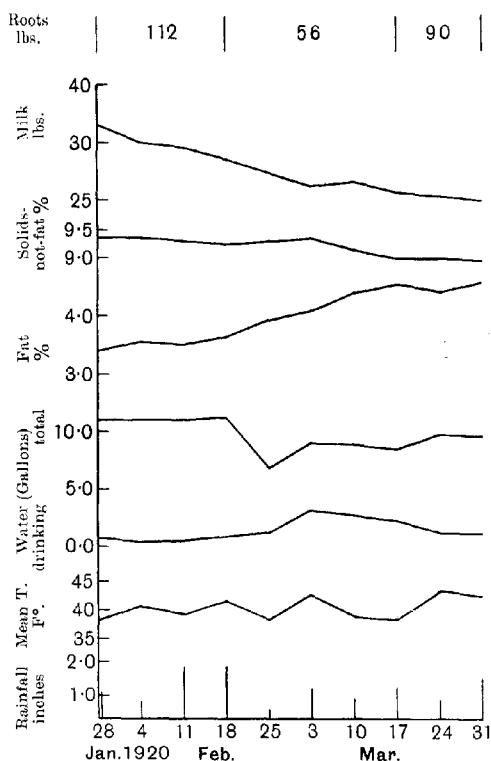


Fig. 4.

The percentage of fat showed a small but continuous increase. The increase was more rapid after the change from the heavy root ration. It was greater than could be accounted for by a shrinkage in the milk yield, see Table III, p. 93, although the change of feeding did not alter the supply of nutritive material as calculated by the usual method.

The percentage of solids-not-fat remained almost stationary although there was a fall in the milk yield. The actual weight diminished, but more rapidly under the heavy compared with the moderate root ration, see Table III below.

The proportions of proteins and of solids-not-fat are found to increase as the lactation advances<sup>1</sup>. On the other hand Crowther<sup>2</sup> finds that the percentage of sugar and solids-not-fat decreased while the proteins increased as the lactation advances.

TABLE III.

	Jan.	February					March				
	28th	4th	11th	18th	25th	3rd	10th	17th	24th	31st	
Average daily milk yield in lbs.,	30.3	29.1	28.7	27.2	26.3	25.2	25.4	25.0	24.8	24.0	
Total solids, %	12.69	12.91	12.72	12.8	13.21	13.4	13.53	13.62	13.31	13.45	
Fat weight in lbs.	1.03	1.06	1.00	0.99	1.02	1.04	1.12	1.13	1.10	1.08	
Solids-not-fat, lbs.	2.83	2.70	2.65	2.49	2.45	2.34	2.32	2.29	2.24	2.16	
Total solids, lbs.	3.86	3.76	3.65	3.58	3.47	3.38	3.44	3.42	3.34	3.24	
Roots, lbs.		112					56		90		

Four out of the seven cows in the experiment took drinking water regularly while on the high root ration and the average quantity taken amounted to 5.1 per cent. of the total intake of water. The water supplied in the roots sufficed for the requirements of the remainder of the cows. The average total water per cow taken daily while on the heavy root ration was 110 lbs., compared with 82 lbs., of which 28 per cent. (see Table IV, p. 94) was drinking water when the cows were fed on a moderate root ration. On the high root ration the proportion of total water to 1 lb. of milk produced was 3.9 lbs. and 3.3 lbs. on the moderate root ration. Table IV, p. 94.

The cows scoured badly while on the heavy root diet and the byre-man complained that their health was being adversely affected. The scouring did not cease until some time after the change of feeding to the moderate root ration. The scouring in this case is the result of feeding excessive quantities of succulent food. The temperature of the water (about 100 lbs.) in the roots would often not be many degrees above that of freezing point in the winter months.

<sup>1</sup> Van Slyke, L. I. and Publow, C. A. *Science and practice of cheese-making*. Orange Judd & Co. New York, 1914, 162. Berry, R. A. "Yield and composition of cow's milk during lactation." *Bull.* 76. W. of Scot. Ag. Coll. Glasgow, 1916.

<sup>2</sup> Crowther, Charles. "Variation in the composition of cow's milk with advance in lactation." *Trans. H. and Ag. Soc. of Scot.*, 1911, 22.

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TABLE IV.

Num- ber of animal	112 lbs. roots				56 lbs. roots				90 lbs. roots					
	Total water consumed				Total water consumed				Total water consumed					
	Milk yield of daily animal lbs.	To lb. of milk produced lbs.	Drink- ing water %	Proper- tion in milk %	Milk yield daily lbs.	To lb. of milk produced lbs.	Drink- ing water %	Proper- tion in milk %	Milk yield daily lbs.	To lb. of milk produced lbs.	Drink- ing water %	Proper- tion in milk %		
2	27.8	116.8	4.2	8.9	20.1	24.1	3.7	34.4	23.3	24.0	105.6	4.4	21.5	21.0
6	32.6	114.1	3.5	6.5	20.7	28.7	3.3	38.2	26.2	29.6	106.5	3.6	22.1	23.8
9	25.6	110.1	4.3	2.3	20.5	23.4	3.5	28.4	24.7	22.3	93.9	4.3	14.8	21.7
11	27.0	108.0	4.0	—	24.2	22.7	3.1	16.2	28.0	20.1	82.4	4.1	5.4	24.3
19	34.1	112.5	3.3	2.9	27.0	29.5	3.1	35.2	28.1	28.4	102.2	3.6	19.0	26.3
24	26.5	106.0	4.0	—	21.8	23.5	3.1	19.5	23.2	22.3	91.4	4.1	9.1	23.8
29	28.2	104.3	3.7	—	25.1	26.3	3.0	26.4	28.4	26.6	90.4	3.4	11.0	25.7
Average	28.8	110.3	3.9	2.9	22.5	25.5	3.3	28.3	26.7	24.8	96.3	3.9	14.7	23.8

The turning out of the cows to pasture in the spring and sometimes when a change is made from ordinary pasture to aftermath, also produces a pronounced laxative effect, but the milk yield invariably shows a marked increase covering a few weeks and accompanied by a perceptible fall in the percentage of milk fat.

A cow on pasturage is generally supposed to eat about a hundred-weight of grass a day. An average sample of grass would supply in lbs.: dry matter, 25; dig. crude proteins, 3.8; dig. carbohydrates and fibre, 12.2; dig. oil, 0.37. Except for the crude proteins there is less nutritive substances than supplied by the heavy root ration used in the foregoing experiment. In the latter case there was a fall in the milk yield and a rise in the fat and the reverse in the former case.

#### TEMPERATURE AND MILK SECRETION.

In an earlier part of this paper (p. 83) it was remarked that a change of temperature might be one of the contributory causes which accounts for the irregular form of the daily milk yield curves. The mean temperature inside the byre in the winter months is, as a rule, from 15° to 20°

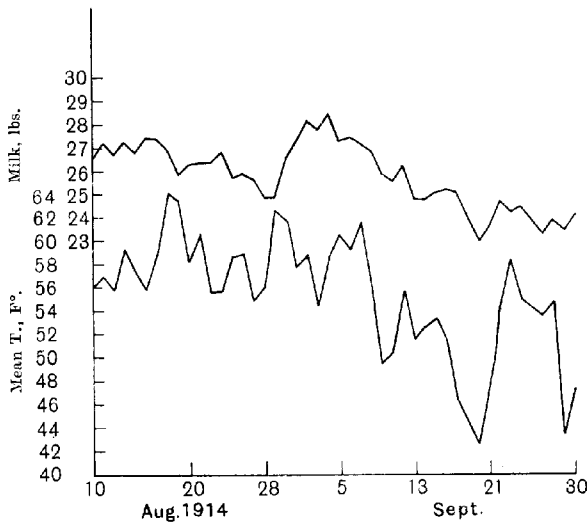


Fig. 5.

higher than that of the outside air and it is subject to much less variation. A curve has therefore been drawn up to show the daily mean

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temperature of the outside air and the average daily yield of milk of cows out all night on pasture in the early autumn. See Fig. 5, p. 95.

From an inspection of the graph for the month of September there appears to be a connection between the curve showing the variation of the milk yield and the curve for the mean temperature. The possibility of this being accidental must be kept in sight in view of the fact that in this month the mean daily temperature tends to fall and the milk yield as a result of advancing lactation diminishes. The rainfall is not included in the graph, but animals at this time of the year, resting over-night on pastures sodden after rain, would almost certainly intensify any effect produced by a falling air temperature.

A similar curve for the winter months giving the mean daily temperature in the byre and in the outside air, and the daily milk yield is shown below (Fig. 6).

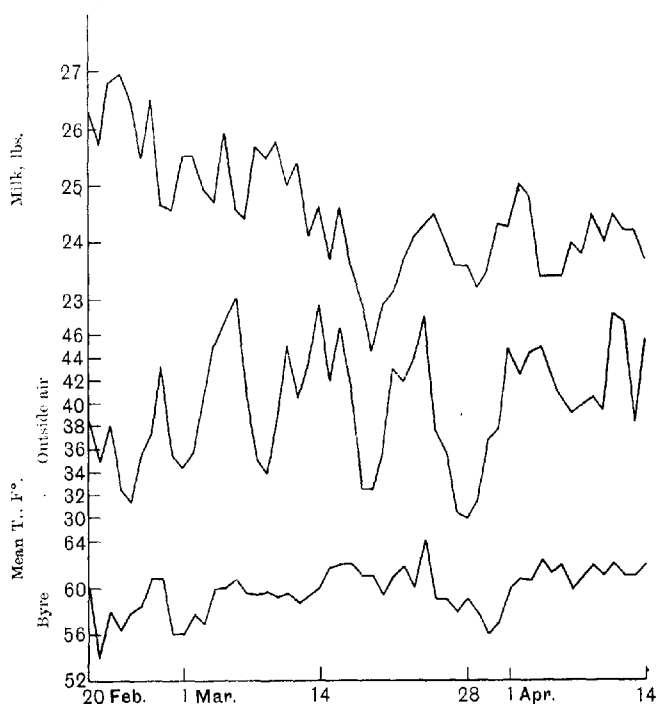


Fig. 6. Byre readings were taken at 9 a.m. and 5 p.m. and the two figures averaged.

In the latter half of the curve there again appears to be a connection between a pronounced change of temperature and milk secretion. A comparison of the milk figures for a period of a comparatively low following or preceding that of a comparatively high temperature, shows that a lowering of the temperature depresses the yield while an increase in the temperature improves the yield. In other cases which were plotted out there seemed to be no connection between the two. Obviously there are other factors concerned, and further data are being obtained.

In an experiment carried out by the Highland and Agricultural Society of Scotland in 1908<sup>1</sup> it was found that the milk yield from cows housed in a byre kept well ventilated and cool was slightly higher compared with that of cows housed in an adjacent byre which was badly ventilated, but warmer. The average temperature of the former was 49.8° F. and for the latter 59.4° F. The purity of the air would not be the same in the two cases.

The effect on the milk fat of a marked fall in temperature has been found to produce a fat with a lower butyric acid content as indicated by the Reichert-Meisel number<sup>2</sup>. The effect on the yield of fat was indefinite. Figs. 1, 2 and 3 should be examined in the light of the foregoing observations.

#### SUMMARY.

The feeding of concentrates after scalding with boiling water and given to cows in winter in a sloppy condition at body temperature, increased the yield of milk by about 1 lb. per head per day over the yield produced when the concentrates were fed in a dry state. The increase diminished with the advance in lactation.

The warm mashies produced a distinct laxative effect.

The animals took to the wet mashies better than to the dry meals.

When the concentrates were fed as dry meals in a ration containing 30 lbs. roots with fodder and the drinking water taken from a trough in the yard, the coat of the animals got out of condition. With a change to the wet meal or when the drinking water was accessible in the byre, the coat regained its condition again.

An improved water supply did not appear to affect the milk yield. The percentage of milk fat increased as the milk yield decreased.

<sup>1</sup> Speir, John. "Influence of temperature on milk yield." *Trans. H. and Ag. Soc. of Scotland. Fifth series*, **21**, 1909, 255-306.

<sup>2</sup> Berry, R. A. "Yield and comp. of cow's milk during lactation." *Bull.* 76. W. of Scot. Ag. Coll. Glasgow, 1916, 49-73.



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Dairy equipment includes a boiler, therefore little, if any, additional capital outlay should be necessary for scalding the meals.

On an average 3.5 lbs. of water were assimilated to 1 lb. of milk produced in a normal winter ration (see p. 89). The ratio varied with the feeding, the temperature and the period of lactation.

Drinking water and water consumed in the food amounted to about ten gallons daily, and of this between 50 and 60 per cent. was drinking water. The amount increased with an increase in the yield of milk and with a rise in the temperature. The smaller cows also consumed a relatively larger proportion of drinking water. From 25 to 30 per cent. of the total intake of water was contained in the milk.

The feeding of a large weight of roots (112 lbs.) with meals and fodder caused the animals to scour badly. The health and the condition of the animal were also adversely affected. The milk yield which had shown a tendency to increase was converted into a definite decrease. There was an increase in the percentage of fat, although the total weight produced diminished. The fall in the milk yield was partly though not immediately arrested by a change to a moderate root ration and there was a decided increase in the percentage of, and in the total yield of, fat. Heavy root feeding would therefore appear to depress fat formation, though the supply of nutritive material in the food as judged by the usual method of calculation was not diminished.

The total weight of water consumed for 1 lb. of dry matter in the food amounted to 3.9 lbs. with the heavy and 3.3 lbs. with the moderate root ration. Some of the cows took water daily amounting on an average to between 5 and 6 per cent. of the total intake of water while on the heavy root ration.

Preliminary data relating to temperature and milk secretion were given.

Ayrshire cows were employed in the experiments.

The late Lieutenant H. A. Wyllie, B.Sc. was in charge of the 1913-14 experiment and Mr Hugh M. Young of the 1914-15 experiment. Determinations of the percentage of total solids in the milk were made by Mr Peter Caldwell.

Any additional expenditure incurred in carrying out these experiments was defrayed by the Board of Agriculture for Scotland.

*(Received November 8th, 1920.)*

OBSERVATIONS ON THE LIFE HISTORY OF THE  
WHEAT-BULB FLY [*LEPTOHYLEMYIA*  
*COARCTATA*, FALL.]

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(With Plates I and II.)

DURING the past fifty years there have been many records of the damage done by this pest to wheat and rye on the continent.

The first record in this country is by Ormerod<sup>(1)</sup> in 1882 in wheat. She says "there is good reason for believing that it was present to a serious extent before." Since that time numerous complaints of serious damage to wheat have been recorded in various parts of the country, but the eastern counties of England and parts of Scotland have suffered most and the intensity of the attack has varied enormously from season to season.

My observations made mostly during the season of 1920 confirm those of other observers that the worst attacks follow a bare fallow or bastard fallow and that bad attacks also occur after crops of potatoes, rape, swedes, turnips and mangold, especially where the soil is bare during the summer, as would obtain with poor crops of roots or where potatoes are dug early or the tops die off early in the season.

In this connection the following communication from Mr F. Hiam, one of our largest fen farmers, is of interest.

WHEAT-BULB FLY.

This pest does not appear to do much harm in a wet, cold or damp summer, but during a dry summer, say from July 20th to September 29th, on all bare lands, namely fallows, early potato land or late potatoes, mangolds and swedes, where there is not sufficient top to keep the sun from the land, the bulb fly will deposit her eggs and if wheat is sown it is sure to be practically spoilt when the eggs hatch in the spring. On wet land, or in a wet season, the eggs do not appear to mature.

No other crops are damaged by this pest and it is quite safe to sow rye, barley or oats on affected land. Should the fallow be clean by the end of July mustard or rape seed could be drilled and then ploughed in for the wheat crop. The same applies to land cleared for early potatoes.

This season, owing to this pest, hundreds of acres of wheat have been destroyed and the land re-ploughed and sown with barley, whilst hundreds of acres are only half crops. Farmers have not been fully acquainted on this subject and a good many light land farmers believe that the sun takes the goodness out of the land; others that it is the wire-worm that spoils the wheat in the spring, but such is not the case and I hope before long the Ministry will find a remedy to stamp out this pest. In the meantime farmers should certainly be advised not to sow wheat on bare land that has been subject to a hot dry summer.

In April, 1920, Warburton recorded its occurrence in winter barley from Essex.

At present it is not known in what stage or where the fly hibernates although several authors suggest that the egg is the wintering stage.

The following experiments throw some light on this.

#### EXPERIMENTAL WORK.

On March 25th a number of wheat plants attacked by wheat-bulb fly larvae were put into two pots of soil in the laboratory. (The larvae were examined to make sure that they were wheat-bulb flies.) In two other pots containing healthy wheat plants wheat-bulb fly larvae were placed.

On May 3rd the pots were examined and 17 pupae were found at a depth of  $1\frac{1}{2}$ -2 inches in the soil.

On April 4th a well-tillered wheat plant with one shoot attacked was placed in a pot and two other larvae added. Another larva was added on April 29th. This pot was examined on May 17th and two pupae removed. I should mention that the larvae used were almost fully grown, judging from their size.

These pupae were placed in moist sand and covered with a breeding cage (a beaker with the bottom out and covered with muslin).

The flies emerged as follows:

June	1st.	1 fly emerged.
	3rd.	3 flies emerged.
	4th.	1 fly emerged.
	5th.	3 flies emerged.
	6th.	1 fly emerged.
	10th.	2 flies emerged.
	11th.	3 flies emerged.

Later (date unrecorded). 1 fly emerged.

These flies were placed in an inverted bell-jar covered with muslin and fed at first on sugar and pepsin which was soon changed to con-

densed milk and meat juice. [As we did not know the natural food of these flies we used the above as likely to supply the food materials necessary for egg-formation.] Moisture and food were at first placed on the muslin top but later supplied by means of a sponge suspended in the bell-jar. Owing to a misunderstanding the first fly which hatched—a male—was killed and set.

*Notes on flies in bell-jar.*

June 25th. 13 flies alive, 1 dead and stuck in condensed milk at the bottom of the jar.

„ 29th. 1 ♂ died.

July 5th. 1 ♂ died.

„ 10th. 1 ♂ died.

On July 8th a clean sponge was suspended in the jar.

On July 14th a number of eggs were found laid in this sponge and also a few in the debris at the bottom of the jar. The flies were transferred into a fresh jar with a layer of sifted soil at the bottom. At 4.30 p.m. three flies were observed laying eggs, the ovipositor being inserted to its full length in the soil. The eggs laid in the bell-jar were placed on damp filter paper in a petri dish. Those laid in the sponge were kept in the sponge for about a month and then transferred to damp filter paper in a petri dish.

July 16th. Flies transferred to a large cage 27 inches long, 15 inches wide and 24 inches high. The top of the cage was covered with a sheet of glass and the sides with muslin. The bottom of the cage consisted of a shallow box which was filled with soil two-and-a-half inches deep. The soil was sterilized by heating to about 90° C. One-third of the area of the soil was kept as a bare fallow, in the middle portion wheat was sown, and at the other end rye grass plants were transplanted.

July 19th. 1 ♀ died.

Aug. 3rd. 3 ♀s caught on the University Farm placed in the cage.

„ 5th. 2 ♀s died; 10 left but only 8 visible.

„ 6th. 1 dead fly, partly decomposed, found; 8 visible.

„ 8th. 4 p.m. 1 fly observed laying eggs in bare fallow; eggs found at a depth of one-eighth inch in soil.

„ 9th. 1 ♀ died; 7 visible.

„ 11th. 6.40 p.m. 1 fly laying eggs in bare fallow; 6 visible.

„ 13th. 1 ♀ died; 3 visible.

„ 14th. Another fly partly decomposed found; 3 visible.

Aug. 22nd. 6 p.m. 1 fly observed laying eggs in loose soil between the wheat plants.

„ 23rd. 9 a.m. and 4 p.m. Fly observed laying eggs in bare fallow.

Sept. 6th. 1 ♀ died; 2 visible.

„ 19th. 1 ♀ died; 1 visible.

The last ♀ spent most of her time thrusting her ovipositor into the soil; but as no eggs were ever found where she had been, she was dissected on Oct. 15th and 37 eggs were taken from her, all of which proved infertile.

Partly decomposed remains of flies were found when the cage was thoroughly examined, which accounts for the missing flies.

Flies which died were soon attacked by mites probably introduced with the rye-grass.

On Sept. 30th the rye-grass plants were removed and carefully examined but no eggs were found on them. Six eggs were found in the soil between the plants.

On Oct. 5th wheat was sown in the fallow and rye-grass parts of the soil, and on Nov. 3rd the wheat was pulled up from the middle plot and resown.

On Nov. 30th a very small wheat plant next to the fallow plot was yellowing and on Dec. 2nd a third stage larva was found to be attacking this plant.

On Dec. 9th I noticed that the centre shoot of one of the wheat plants on the row next the fallow was wilting slightly although still green. An examination of this plant revealed the presence of a *first stage larva* which was feeding at the base of the young shoot. This attack must have started after Nov. 3rd (the time of sowing).

In the field I have found only second and third stage larvae; this is probably because the attack is not noticed before the centre shoot yellows and by this time the larva has moulted. In the plant in which I found the first stage larva the centre shoot was not yellow.

[N.B. In order to prevent the plants from yellowing through lack of food the soil was watered at intervals with a plant food solution consisting of superphosphate, potassium nitrate, magnesium sulphate and a trace of ferric chloride.]

## EGGS.

The eggs which are flattened at one end measure 1.25 mm. in length and .5 mm. in width. They are creamy white, shining, and apparently smooth but under the microscope they show longitudinal ridges as shown in Pl. I, fig. 1.

The eggs were kept on damp filter paper in a petri dish in a room on the ground floor on the north side of the building. Some were kept fairly moist and others were kept fairly dry at first, but later when the moister ones began to hatch, these were also kept fairly moist. The only treatment which the eggs received was in cases where a fungus was found growing on them: they were then brushed with a camel-hair brush.

## LARVAE.

In dish 1 (kept fairly moist) *three* larvae hatched on Sept. 11th. These were kept for descriptive work.

There was a long pause before the next hatch which took place on Nov. 24th. Two hatched in dish 1 but the larvae were very weak when found in the morning and remained without moving all day, so they were mounted.

On the same day in dish 2 (kept dry at first and moist later) one larva got nearly half way out of its egg but was unable to get further.

An examination of these larvae showed two types, very similar; but differing in spiracles, arrangement of tracheae, in the size of their basal papillae and with a slight difference in the mouth-parts. Keilin says "these types belong to different flies, probably very closely allied."

If this is so, then there are two species of wheat-bulb fly, as the flies which laid these eggs were all reared from maggots found attacking wheat. Unfortunately some of these flies were rendered unfit for identification after dying in the cage and before they were found.

The remainder of the flies taken from the cage were all identified by Collin as *L. coarctata*.

At the time of writing (Dec. 10th) some of the eggs are still apparently normal.

On Dec. 7th I placed them in a cool room in the basement to retard the hatching which has most probably taken place much too early under the abnormally warm conditions under which the eggs have been kept.

Kurdjumov(2) gives a description of the egg and first stage larva of

*L. coarctata* but his figure of the egg is very different from mine (see Pl. I, fig. 1).

Keilin(3) has already pointed out that the mouth-parts of Kurdjumov's first stage larva pertain to those of a carnivorous larva and concludes that it does not belong to the developmental cycle of *L. coarctata*.

The mouth-parts of all my first stage larvae, which are very different from those described by Kurdjumov, correspond very closely with the second and third stage larvae (Pl. I, figs. 2, 3, Pl. II, figs. 4 and 5).

#### LIFE HISTORY.

My experiments and observations confirm the supposition of several observers that *L. coarctata* has only one generation per annum in this country. Comte(4) records two generations on wheat in Tunisia; and Wahl(5) records two generations in Germany but only one in Denmark.

The life history of *L. coarctata* as deduced from my experiments and observations is as follows:

The flies hatch out in June and July and lay their eggs in bare soil about one-eighth of an inch below the surface in July, August and possibly September. Most of these eggs hatch out early in the following spring as they are usually found in the wheat plants in March and April. A few may, however, hatch out the same autumn as on Nov. 23rd, 1917, I found two third stage larvae attacking wheat plants on the University Farm. On Feb. 13th, 1920, I found a second stage larva attacking wheat on the University Farm.

The larva on hatching from the egg makes its way into the middle of a wheat shoot, where it feeds at the base of the shoot which it kills. Wahl(5) has shown that the larvae may attack several plants in succession. When fully fed the third stage larvae make their way into the soil where they pupate about one-and-a-half to two inches below the surface. Pupation usually takes place in May.

This habit of egg-laying in bare soil in the case of a specific pest seems to be unique and may possibly be due to a change of diet. It will be interesting to find out what happens when crops other than cereals are sown in soil containing their eggs.

My thanks are due to Dr Keilin for his help in identifying the different larval stages; to Mr J. E. Collin for identifying the flies; and to Mr D. Boyes for his help in carrying out the experiments and observations.

Dr Keilin has promised to publish a detailed description of the larval stages later.

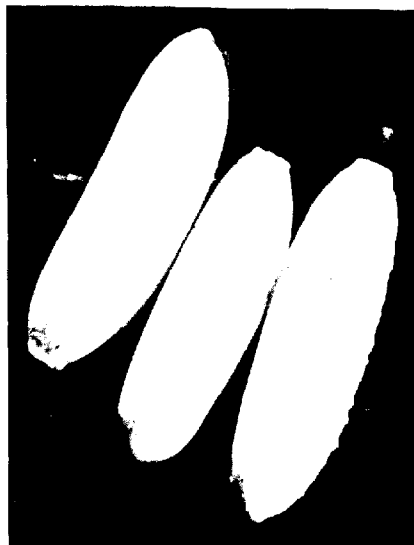


Fig. 1

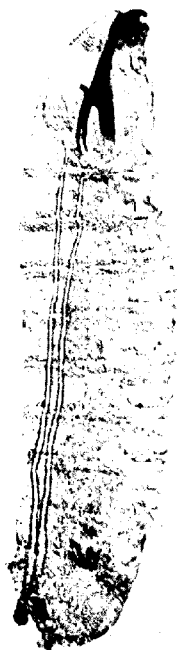


Fig. 2



Fig. 3







Fig. 4





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## EXPLANATION OF PLATES I. and II.

## PLATE I.

- Fig. 1. Eggs of *Leptohylemyia coarctata*. (The longitudinal ridges seen at the rounded end of the middle egg are present throughout the whole length of the egg.)
- Fig. 2. First stage larva of *L. coarctata* (one of the types reared from an egg on filter paper). (Actual length 1.47 mm. much flattened by the cover-slip.)
- Fig. 3. Mouth-parts of same.

## PLATE II.

- Fig. 4. Anterior end of second stage larva (same magnification as Fig. 2).
- Fig. 5. Anterior end of third stage larva (same magnification as Fig. 2).

(Received 11th December, 1920.)







## STUDIES IN CROP VARIATION.

I. AN EXAMINATION OF THE YIELD OF DRESSED GRAIN  
FROM BROADBALK.

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(With Three Figures in Text.)

## I. THE VARIATION IN WHEAT YIELD.

1. *Introductory.*

THE crop records available at Rothamsted extend back for over 70 years. In Broadbalk wheat field 13 plots have been continuously under uniform treatment since 1852. The length of this unique series of observations is of special value for statistical purposes. The average yields obtained from the Board of Agriculture's Reports cover only half the period; even the Woburn records date only from 1877. The series of yields of dressed grain from the different plots of Broadbalk was examined from 1852, when uniform treatment commenced, until 1918, the last season available when the examination was begun.

Several minor alterations have been made from time to time in the manurial treatment, even in the selected plots, but in the main it has remained uniform.

(a) For the first 7 years plots 5, 6, 7, 8, 13, and 17 or 18, received 300 lbs. per acre of sulphate of potash, of which for the subsequent 60 years they received 200 lbs. per acre. At the same time the sulphate of soda was cut down from 200 to 100 lbs. per acre on plots 5, 6, 7, 8, 16, and 17 or 18, and from 550 to 366½ lbs. per acre on plot 12; while the sulphate of magnesia on plot 14 was reduced from 420 to 280 lbs. per acre.

(b) For two years, 1862 and 1863, all plots, except half of plot 3 received 400 lbs. per acre of a mixture of silicates of soda and lime, and for 3 subsequent years, one half of plots 5, 6, 7, 8, 17 or 18 received 228 lbs. per acre of this mixture. A comparison of the corresponding



sub-plots shows that this dressing had little effect, being, if anything, deleterious to the sub-plots receiving it.

(c) For 12 years, 1868 to 1879, the chopped straw from each sub-plot was ploughed in, on half of plots 5, 6, 7, 8, 11, 12, 13, 14, and 17 or 18. A comparison of the corresponding sub-plots shows that in this case also the effect upon the mean yield may be ignored in the analysis of the majority of the plots. Plots 11, 12, and 14 in which the deficiency of potash is a limiting factor showed a sensible benefit from the straw, and for these series (without straw) has been used from 1868.

(d) For 5 years, 1898 to 1902, a dressing of 400 lbs. per acre of basic slag was substituted for the previous dressing of 392 lbs. of superphosphate, on all plots receiving phosphate. No statistical evidence can be adduced as to the effect of this change, and it is assumed that the dressings were effectively equivalent.

(e) For 1916 the supply of potassium sulphate was reduced to two-thirds of its previous dressing, the ordinary amount of potash being made up by the use of wood ashes. In 1917 and 1918 potassium sulphate was omitted altogether from all the plots, and owing to the insufficiency of wood-ash the deficiency was not made up. In order to continue the comparative treatment of plots 12, 13 and 14, the sodium sulphate on 12 and the magnesium sulphate on 14 were omitted for the same year. From 1920 the original dressings have been resumed.

(f) From time to time small areas have been incorporated in, or excluded from, the experimental plots. These changes can have had but little influence upon the yield per acre, which has always been reckoned on the actual area of each plot; the changes in size have not been large, and any new portion has received similar treatment to that of the main plot, for some years before incorporation.

#### *Changes of Variety.*

The variety of wheat seed employed has not been kept the same. The following varieties are recorded:

1852. Old Red Cluster.

1853-81. Red Rostock, 29 years.

1882-99. Red Club, 18 years.

1900-1916. Squarehead's Master, 13 years.

1917 onwards. Red Standard.

Between 1900 and 1912 the varieties were changed more frequently. Squarehead's Master has been principally used, but intermitted with Giant Red (1905), Browick Red (1910), Little Joss (1911, 1912). Red

Standard was adopted in 1917 and will be used in future. When the varieties are changed infrequently, any effect due to genetic difference of constitution would be included in the slow changes. During the latter period it would appear partly as annual variation. That these genetic differences are not at any rate a principal cause of the slow changes observed, may be seen from the great changes in mean yields which occurred during the use of Red Rostock.

### 2. *The causes of variation in wheat yields.*

From the series of observations it is possible to distinguish three types of variation in the wheat yield: (1) annual variation, (2) steady<sup>1</sup> diminution due to deterioration of the soil, (3) slow changes other than steady diminution. The annual variations may be ascribed primarily to the weather; including in that term not only the direct effects of meteorological conditions in stimulating plant growth, but also the physical effects wrought upon the soil, such as the washing out of plant nutrients and the indirect effects of light, temperature and moisture in stimulating or retarding the increase of bacteria, protozoa and of the fungal and algal flora of the soil, all of which may be supposed to adjust their activities rapidly to the meteorological conditions. The steady diminution of yield may unhesitatingly be ascribed to deterioration of the soil; either, as in plot 10, to the exhaustion of natural supplies of potash and phosphorus, or, in other cases, perhaps, to that of unknown substances required in small quantities, and not supplied in the artificial manure, or to physical changes as yet but little understood, or, in plot 5, to the gradual exhaustion of the power of the soil of producing nitrates in the soil moisture. The third class of change is unexpected, and it is not easy to assign it entirely to any one cause. To establish the existence of large changes in the mean yield, to show how they may be disentangled from the other types of change, and to suggest their possible cause is the purpose of the present paper.

### 3. *Mean Yield and Annual Diminution.*

In Table I is shown the mean yield and average annual diminution of mean yield, in bushels per acre, for the 13 plots here considered.

<sup>1</sup> The deterioration must not be assumed to be mathematically linear, although it is here represented by a linear function; on most plots it was probably more rapid in the earlier years than it was later, as is indicated by the parabolic term being on the whole more inclined to positive values on those plots in which the deterioration is more rapid. The true curves of deterioration cannot, however, be disentangled from the slow changes which have taken place owing to other causes.

Table I.

Plot	Manure per acre						Mean (Bushels per acre)	Mean annual diminution (Bushels per acre)	P. for deterioration
	Sulphate of potash lbs.	Sulphate of soda lbs.	Sulphate of magnesia lbs.	Super- phosphate lbs.	Sulphate of ammonia lbs.	Chloride of ammonia lbs.			
2b, dung 14 tons	—	—	—	—	—	—	34.549	·031	·41
3 and 4, no manure	—	—	—	—	—	—	12.269	·097	·000,001,4
5	200	100	100	392	—	—	14.180	·090	·000,000,8
6	200	100	100	392	100	100	22.581	·141	·000,11
7	200	100	100	392	200	200	31.367	·144	·002,1
8	200	100	100	392	300	300	35.684	·092	·056
10	—	—	—	—	200	200	19.504	·157	·000,25
11	—	—	—	392	200	200	22.046	·219	·000,003
12	—	366½	—	392	200	200	28.319	·181	·000,35
13	200	—	—	392	200	200	30.209	·123	·009,1
14	—	—	280	392	200	200	27.765	·231	·000,000,6
17, alter-	200	100	100	392	—	—	14.510	·092	·002,8
18, nate	—	—	—	—	200	200	29.006	·114	·005,6

In the last column, P represents the probability of a larger annual diminution occurring by chance owing to the later seasons happening to be on the average less favourable than the earlier ones. In calculating P it has been assumed that there has been no real deterioration of the average weather, an assumption which is tested in Section 11.

The values show that all but the dunged plot, 2b, have suffered sensible deterioration. Even plot 8, which receives "complete" artificial manures, shows a deterioration which would not be expected more than once in eighteen random trials. It is, therefore, probably real, as are certainly all the others.

#### 4. *Slow changes in mean yield.*

It becomes apparent on inspection of the actual yields that the changes in mean yield are by no means fully expressed as simple deterioration. The mean yield rises up to about 1860, and after a bad period in the seventies reaches a second maximum in the nineties. The probability of such large fluctuations occurring by chance may be calculated by the methods of Section 8, on the assumption, as above, that the incidence of good and bad seasons has not been orderly but fortuitous.

When the variation of any quantity (variate) is produced by the action of two or more independent causes, it is known that the variance produced by all the causes simultaneously in operation is the sum of the values of the variance produced by each cause separately. The variance is defined as the mean square deviation of variate from its mean, and is therefore the square of its standard deviation. The above property of

the variance, by which each independent cause makes its own contribution to the total, enables us to *analyse* the total, and to assign, with more or less of accuracy, the several portions to their appropriate causes, or groups of causes. In Table II is shown the analysis of the total variance for each plot, divided according as it may be ascribed (i) to annual causes, (ii) to slow changes other than deterioration, (iii) to deterioration; the sixth column shows the probability of larger values for the variance due to slow changes occurring fortuitously.

Table II.

Plot	Annual causes	Slow changes	Deterioration	Total	P. for slow changes
2b	33.2	17.6	.4	51.1	.000,002
3 and 4	9.3	2.5	3.5	15.4	.004,3
5	11.7	2.7	3.0	17.5	.007,5
6	30.6	8.0	7.5	46.1	.003,1
7	50.3	13.3	7.8	71.4	.001,8
8	53.2	15.9	3.2	72.3	.000,58
10	41.8	3.7	9.2	54.7	.26
11	50.2	4.2	18.0	72.4	.27
12	58.4	7.8	12.2	78.4	.086
13	50.7	8.3	5.7	64.6	.040
14	49.2	6.3	20.0	75.4	.10
17) minerals	21.4	4.6	3.1	29.1	.011
18) ammonia	38.2	9.0	4.9	52.1	.005,4

The majority of the plots could be cited independently as evidence that the slow changes in the mean yield are not fortuitous. The less significant values from plots 10 to 14 are due not so much to the relative absence of slow changes, as to the great sensitiveness of these plots to annual causes of variation.

The great variability of P is itself evidence that the slow change effect is of a different origin from that of the annual causes, for P is calculated from the ratio between these two portions. If, for instance, progressive alterations had been taking place in the average weather to an extent unmistakably distinct from independent annual variations occurring in random order, then we should expect all plots to show very small values of P, whereas, in fact, P is greatest on those plots most subject to meteorological disturbance; on the other hand, if, as appears to be the case, the succession of seasons is not distinguishable from a random order, then the very small values of P observed in some plots must have some explanation other than meteorological.

The actual course and extent of the slow changes in mean yield, other than soil deterioration, is best shown by a smooth curve, a polynomial of the 5th degree, fitted to the series of yields of dressed grain

from plot 2b (Fig. 1a). This plot is not only sensibly free from deterioration but, as shown by the value of P, is least affected by accidental fluctuation due to good and bad seasons. In order to obtain data for the study of meteorological effects, it was necessary to eliminate as far as possible

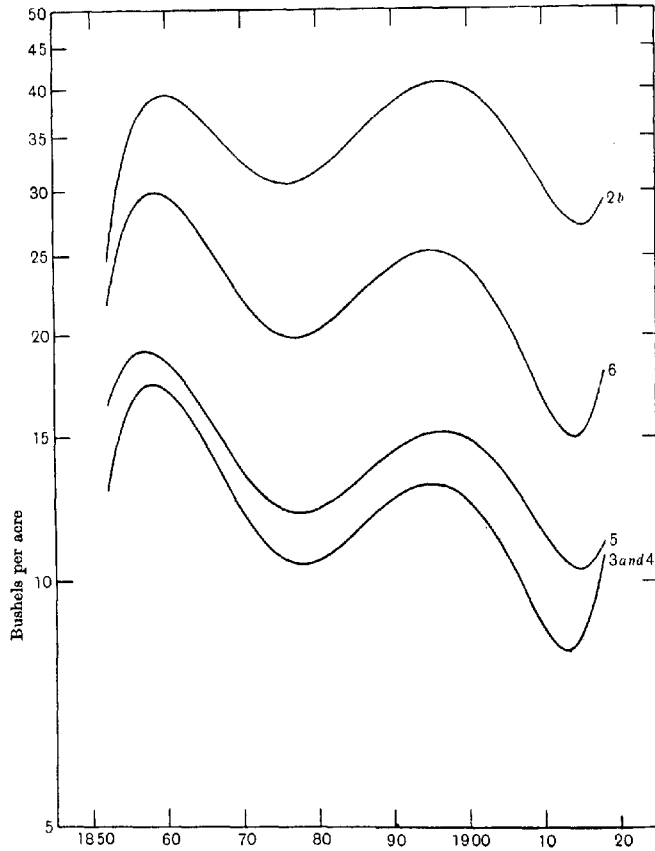


Fig. 1a.

Fig. 1. Showing the course of the changes in mean yield in the continuously manured plots of Broadbalk. The vertical ordinate is plotted on a logarithmic scale in order to shew the proportionality of the slow changes in the several plots, and in order that the relative importance of soil deterioration may be compared.

variations of other than meteorological origin; for this purpose similar curves were fitted to all the plots, and the annual deviation of each plot, from the corresponding curves, will form the basis of a study of the

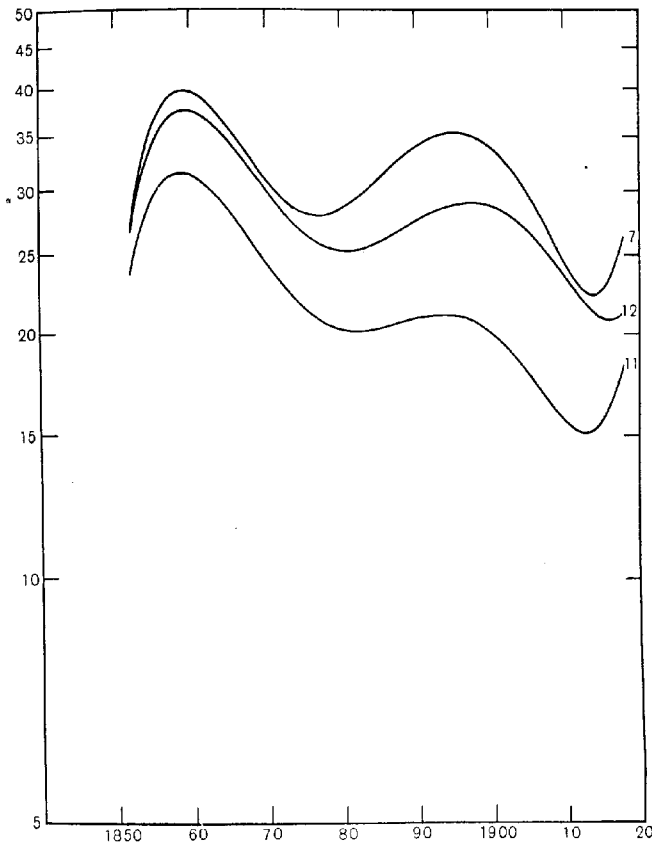


Fig. 1b.

effects of weather upon wheat yield. Fig. 1 shows that during two periods of high yields, centred about 1860 and 1897, the mean yield of plot 2b was about 40 bushels per acre, while in the depression about 1876 it had fallen nearly to 30 bushels per acre, and in 1915 even lower. For special

reasons (Section 9) the extreme values are not so reliable as the others; yet there can be little doubt that the recent minimum is already past, and that a genuine improvement took place in the early years of the experiment.

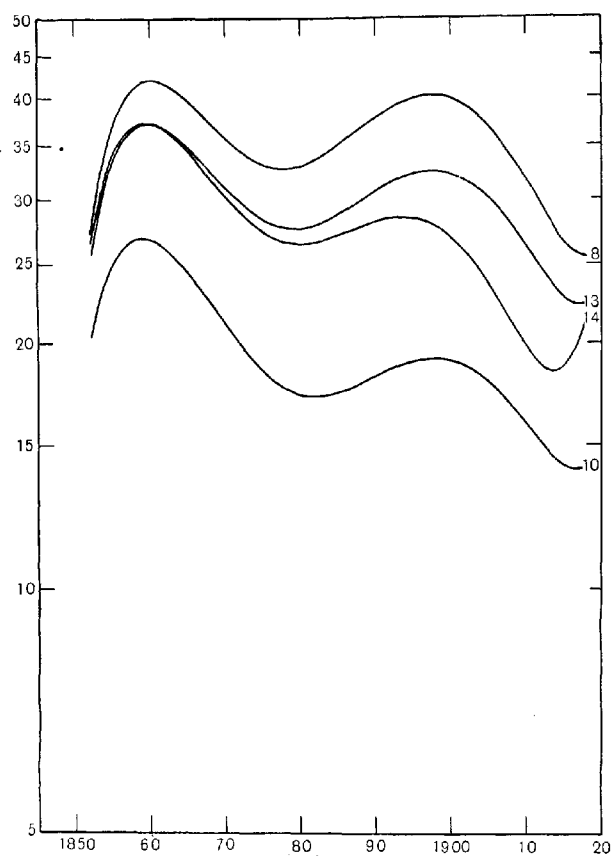


Fig. 1c.

5. *Yield and exhaustion in relation to manurial treatment.*

Before passing to the theory of the significance of these polynomials attention may profitably be drawn to the effect of different manurial treatment upon the mean yields and average annual decrements in the different plots. The probable errors refer in each case to the deviations to be expected from variations of weather occurring in random order.

Tables III, IV, V and VI give the mean yield with the absolute and relative rate of decrement arranged to show best the effect of differences of manurial treatment.

Table III.

Plot	Mean yield (Bushels per acre)	Mean annual decrement (Bushels per acre)	Mean annual decrement %
5, no ammonia	14.18 $\pm$ .44	.090	.63 $\pm$ .16
6, single ammonia	22.58 $\pm$ .71	.141	.62 $\pm$ .19
7, double ..	31.37 $\pm$ .90	.144	.46 $\pm$ .15
8, treble ..	35.09 $\pm$ .93	.092	.26 $\pm$ .14

Plots 5, 6, 7 and 8 all receive the same dressing of superphosphate and of sulphates of potash, soda and magnesia, they differ by successive units of ammonium sulphate. The first increment is applied, half in autumn and half in spring, the subsequent increments in spring only. The increases in mean yield due to these increments of ammonium sulphate are 8.40, 8.77 and 4.34 bushels per acre, a series suggestive of "diminishing returns" although not conforming to the geometric series which has been proposed for such cases (Mitscherlich, 1909). The figures for the mean annual decrement, in the second column, show that although the mean of plot 6 exceeds that of plot 5 by 8.4, the latter plot has gained on 6 during the 67 years under discussion, at the rate of .051 per annum. At this rate the difference would disappear in 165 years. Naturally no such result is anticipated from such a prolonged experiment; but this consideration serves to show that not only the mean values, but the rates of decrement observed, are comparable only when taken over the same period of years.

It is to be expected that if the experiment were continued indefinitely, each plot would approach continually to a constant mean yield, but that these yields would differ materially for different manurial treatment. An approximation to the relations which finally exist between the mean yields of different plots may be obtained from the mean of the last nine years of the polynomial fitted to each plot. The values obtained are



10·77, 15·97, 23·57 and 30·16, for these four plots, and the differences produced by the three successive increments are 5·20, 7·60 and 6·59. In these figures there is no longer any evidence of "diminishing return." The comparative yields of these four plots have become the standard example of diminishing returns in agriculture, and in the earlier years of the experiment fully bore out the anticipations of the economists. For the first 9 years of the polynomial, for example, the successive increments were 9·27, 8·15 and 1·43, and we have seen that a similar effect, though less marked, still appears in the means of 67 years. It is only in recent years that the progressive deterioration of the less highly manured plots has gone so far as to make the third increment of the series exceed the first, and so to make apparent the fact that the benefit of the higher dressings was not wholly reaped in the immediate yield, but to some extent is long effective in maintaining the fertility of the soil at a higher level.

The average annual decrements when set out as percentages of the mean yield show a progressive advantage of the nitrogenous manuring. Since all the plots have received soda, potash, magnesia, sulphates and phosphates, equally while greater weights of these ingredients have been removed from the more highly manured plots, the advantage of the latter may be safely ascribed to the supply of nitrogenous plant nutrients. The increasing effectiveness of the nitrogenous manure might be ascribed to the gradual utilisation of slowly available nitrogen compounds in the soil; this view, however, is not borne out by the comparative nitrogen analysis of these plots, which are available since 1865, and it is more probable that the heavier vegetation supported by plot 8 has had some more indirect beneficial effect, equivalent to a more ample supply of nitrogen. The greater root growth on the heavy yielding plots has perhaps increased the effective depth of soil activity, or has supplied more abundantly substances required for bacterial life. If the natural supplies of nitrogen in plots 5 and 6 have become impoverished in these ways we should expect to find the additional dressings of sulphate of ammonia to become, as they have become, an increasingly important factor in maintaining yield.

The four plots (3 and 4), 5, 10 and 7 illustrate the supplementary action of nitrogenous compounds on one side and mineral manures on the other. The mean yield of 7 not only exceeds that of 10 by a greater amount than does 5 exceed (3 and 4), but it exceeds it in a higher ratio. In its absolute rate of decrement 10 exceeds 7 and (3 and 4) exceeds 5, although in both cases giving a lower mean yield. This can only mean

that a part of the decline of (3 and 4) and 10 is to be ascribed to the progressive exhaustion of some of the mineral ingredients supplied to 5 and 7.

Table IV.

Plot	Mean yield	Mean annual decrement	Annual decrement %
3 and 4, no manure	12.27 $\pm$ .39	.097	.79 $\pm$ .16
5, minerals ...	14.18 $\pm$ .44	.090	.63 $\pm$ .16
10, ammonia ...	19.50 $\pm$ .83	.157	.81 $\pm$ .22
7, ammonia and minerals ...	31.35 $\pm$ .90	.144	.46 $\pm$ .15
17) minerals ...	14.51 $\pm$ .59	.092	.63 $\pm$ .21
18) ammonia ...	29.01 $\pm$ .79	.114	.39 $\pm$ .14

The comparison with the two series from plots 17 and 18 shows that the mineral series is very little better than 5, and much worse than 7, showing that there is little residual effect of the ammonium salts applied the previous year. That this small advantage is accompanied by greatly increased variability appears from Table II, in which the variance due to annual causes in plot (17 and 18) minerals is nearly double that of plot 5; from the considerations of Section 14 this may be due to the greater effects in variations of weed prevalence. The relative annual decrement also agrees with 5, in being much greater than 7. On the other hand, the ammonia series of (17 and 18) has a mean yield much more close to 7 than to 10, showing that there is relatively little ultimate loss of the mineral ingredients applied in previous years. The relative decrement of (17 and 18) ammonia is even less than that of 7, owing presumably to the smaller crops, averaging only about 21.8 bushels per acre, which have been taken off these plots. The difference in annual decrement, .07 per cent., is very small compared with the difference in mean yield, of 9.6 bushels per acre; and this suggests that, in this case also, if residual minerals could be tested year after year against minerals freshly applied, the higher yielding series would display the less deterioration.

Table V.

Plot	Mean yield in bushels per acre	Mean annual decrement	Annual decrement %
11, no K, Na or Mg ...	22.05 $\pm$ .91	.219	.99 $\pm$ .21
12, sulphate of soda ...	28.32 $\pm$ .98	.181	.64 $\pm$ .18
13, sulphate of potash ...	30.21 $\pm$ .91	.123	.41 $\pm$ .16
14, sulphate of magnesia ...	27.76 $\pm$ .90	.231	.83 $\pm$ .17
7, All three sulphates ...	31.37 $\pm$ .90	.144	.46 $\pm$ .15

In comparing plots 7 and 13 it is surprising that the latter, which has slightly the lower mean yield, should have the lower relative rate

of decrement. This fact by itself suggests that the addition of sulphates of soda and magnesia to the sulphate of potash, stimulates production, perhaps by making more potash available, but causes more rapid exhaustion. On the other hand, if we contrast either 12 or 14 with 11, the actual production is much increased by the addition of sulphate of soda or magnesia and the relative annual decrement is reduced.

In these figures it is not easy to distinguish any physical effect of the large quantities of saline matter annually added to the plots. In comparing plot 7 with 13 the former receives a large quantity of sulphates of soda and magnesia, which, in view of the dressings of sulphate of potash which both receive, might be expected to add little to the available plant nutrients while adding much to the salinity. That the average yield of plot 7 exceeds that of plot 13, suggests that even in this case the nutrient advantage outweighs the effect of additional salinity, and it is not clear that the greater deterioration of plot 7 is to be ascribed to the latter cause, since this is also characteristic of plots 12 and 14 in which it must be largely ascribed to the progressive exhaustion of soil potash, which the sulphates of soda and magnesia have naturally facilitated.

Table VI.

Plot	Mean yield in bushels per acre	Mean annual decrement	Mean annual decrement %
2b	34.55 $\pm$ .74	.031	.09 $\pm$ .11
8	35.09 $\pm$ .93	.092	.26 $\pm$ .14

The dunged plot 2b can only be well compared with 8. Alone of the plots it shows no significant diminution of yield; plot 8 comes third in this respect, the probability of an increment or decrement as large or larger being the result of a random distribution of favourable and unfavourable years being .056 (or 17 to 1) for 8, against .41 (nearly even odds) for 2b. On the average of 67 years 8 gives the higher mean, but the difference, 1.14, is less than 2b gains on 8 during 20 years, so that during the present century 2b has had the highest mean yield. In addition to having the best sustained, and now the highest yield, 2b is the least variable; the standard error of the annual decrement per cent. in the other plots ranges from .14 to .22, in 2b it is .11. The crop on the dunged plot is therefore better placed to do moderately in a bad year of drought or excessive rain, than to take great advantage of a favourable season.

## II. THEORY OF POLYNOMIAL FITTING.

6. *Uncorrelated terms  $T_r$ .*

If a quantity  $x$  have values  $x_1, x_2, \dots, x_n$  at a number  $n$  of successive times, the general course of its changes may be represented by a polynomial,

$$a + bt + ct^2 + \dots + kt^r,$$

in which  $t$  represents the time. In this expression the coefficients  $a, b, c, \dots, k$ , will be altered if more or fewer terms of the series be used; for instance, the value of  $a$  will be different according as one takes 2 or 3 terms, for unless  $c$  is zero the mean value of  $ct^2$  will not be zero. If the coefficients obtained are to be independent of the number of terms employed, the successive terms must consist of polynomials of degree 0, 1, 2,  $\dots, r$ , which are mutually uncorrelated. Such uncorrelated polynomials may be obtained uniquely in succession, for the term of degree  $r$  must fulfil  $r$  conditions in order to be uncorrelated with the preceding terms; these conditions specify the term completely with the exception of a numerical factor which is absorbed in the coefficient.

When the values, as do annual numbers, stand at equal intervals of time,  $t$  may be conveniently measured from the mid-point of the series, in units equal to the time interval; the series is then

$$A + Bt + C(t^2 - n_2) + D\left(t^3 - \frac{n_4}{n_2}t\right) + E\left\{t^4 - \frac{n_6 - n_2n_4}{n_4 - n_2^2}t^2 + \frac{n_2n_6 - n_4^2}{n_4 - n_2^2}\right\} \\ + F\left\{t^5 - \frac{n_2n_8 - n_4n_6}{n_2n_6 - n_4^2}t^3 + \frac{n_4n_8 - n_6^2}{n_2n_6 - n_4^2}\right\}$$

as far as  $t^5$ .  $n_2, n_4, n_6$ , and  $n_8$  represent the mean value of  $t^2, t^4, t^6$ , and  $t^8$ , and for a series of  $n$  terms,

$$n_2 = \frac{1}{12}(n^2 - 1),$$

$$n_4/n_2 = \frac{1}{20}(3n^2 - 7),$$

$$(n_6 - n_2n_4)/(n_4 - n_2^2) = \frac{1}{14}(3n^2 - 13),$$

$$(n_2n_6 - n_4^2)/(n_4 - n_2^2) = \frac{3}{880}(n^2 - 1)(n^2 - 9),$$

$$(n_2n_8 - n_4n_6)/(n_2n_6 - n_4^2) = \frac{5}{18}(n^2 - 7),$$

$$(n_4n_8 - n_6^2)/(n_2n_6 - n_4^2) = \frac{1}{1008}(15n^4 - 230n^2 + 407).$$

*Variance accounted for by each term.*

The coefficients are chosen so as to make the residual variance a minimum; consequently the residual variance is reduced at each stage by an amount equal to the variance of the term itself. If  $T_r$  stand for

the term of degree  $r$ , the mean value of  $T_p T_q$  is necessarily zero, when  $p$  and  $q$  are different, while the mean value of  $T_r^2$  is

$$\frac{(|r|)^4}{[2r][2r+1]} (n^2 - 1^2)(n^2 - 2^2) \dots (n^2 - r^2).$$

Thus  $T_1^2 = \frac{n^2 - 1}{12},$

$$T_2^2 = \frac{(n^2 - 1)(n^2 - 4)}{180},$$

$$T_3^2 = \frac{(n^2 - 1)(n^2 - 4)(n^2 - 9)}{2800},$$

$$T_4^2 = \frac{(n^2 - 1)(n^2 - 4)(n^2 - 9)(n^2 - 16)}{44,100},$$

$$T_5^2 = \frac{(n^2 - 1)(n^2 - 4)(n^2 - 9)(n^2 - 16)(n^2 - 25)}{698,544}.$$

The variance contributed by each term, and by which the residual variance is reduced when that term is removed, is therefore of the form  $B^2 T^2$ ,  $C^2 T^2$ , and so on.

#### 7. Distribution of variance for unchanging series of independent values.

A series may be said to change when the chance of an observation falling in any given range is a function of the time. When this is not the case the series is unchanging. The main difficulty in the adequate treatment of annual returns of economic and vital statistics lies in the prevalence of profound changes in the population observed. It is probable that the disturbing factors could be largely eliminated by the discriminating use of polynomials as here described.

If  $A_p$  is the coefficient of the term of the  $p$ th degree, and  $x$  is any observed value, then  $A_p = \overline{T_p x / T_p^2}$ ; hence the variance of this term

$$A_p^2 \overline{T_p^2} = \overline{T_p x^2 / T_p^2},$$

if the values of  $x$  are independent, each with standard deviation,  $\sigma$ , and the series unchanging

$$A_p^2 \overline{T_p^2} = \frac{\sigma^2}{n}.$$

Hence, if polynomials are fitted to unchanging series of  $n$  quantities, the variance contributed by each term is on the average  $1/n$  of the total variance. After fitting  $n$  terms the fit is necessarily perfect, and the residual variance zero. The first step merely consists in ascertaining the mean, as is ordinarily done before finding the variance, the subsequent

*curve fitting, applied to an unchanging series divides the variance up into  $(n - 1)$  parts, each of which has on the average the same value.*

The actual frequency distribution of these fractions may be most simply shown by putting  $\epsilon_p^2$  for the variance of the term of degree  $p$ ;  $\epsilon_p$  being supposed +ve or -ve according to the sign of  $A_p$ . Thus

$$\epsilon_p = A_p \sqrt{\overline{T_p^2}} = T_p x / \sqrt{\overline{T_p^2}}.$$

If  $x$  is distributed normally about its mean value, so then is  $\epsilon_p$  with a standard deviation, which we have already evaluated at  $\sigma/\sqrt{n}$ . In unchanging series every value of  $\epsilon_p$ , except the first, is distributed about zero as its mean;  $\epsilon_0 = \bar{x}$ , the mean of the series.

In a changing series in which the change consists mainly in an alteration of the mean value, we may represent the change in mean value by a sufficient number of terms of a polynomial, the deviations from the mean being then an approximately unchanging series. The mean value of  $\epsilon_p$  is then the value corresponding to the series of means; its standard deviation is unaltered, provided that  $\sigma$  is interpreted as the standard deviation of any observation from the changing mean.

#### 8. *The significance of an observed term.*

If the change in the mean value is sufficiently represented by the terms up to  $T_r$ , then the mean value of the residual variance is

$$\frac{n - r - 1}{n} \sigma^2.$$

If the residual variance is  $\sigma_r^2$  we may therefore take

$$v = \frac{\sigma^2}{n} = \frac{\sigma_r^2}{n - r - 1},$$

as the variance of each  $\epsilon$  obtained. It may be that  $\sigma_r^2$  is slightly increased if the terms up to  $T_r$  do not fully represent the course of the mean, in this case the significance of the preceding terms may be somewhat underestimated. There is no tendency to overestimate their significance.

If  $v$  is the mean variance contributed by each term, the mean value of the total variance contributed by  $p$  terms is  $pv$ . If  $t$  is the variance from an actual sample of  $p$  terms, the distribution of  $t$  is easily seen to be  $df \propto t^{\frac{p-2}{2}} e^{-\frac{t}{2v}} dt$ , a curve of type III. The standard deviation of  $t$  is therefore

$$\sigma_t = v \sqrt{2p}.$$

The standard error in the determination of the annual variance  $\sigma^2$  is therefore represented by a coefficient of variation equal to  $100\sqrt{\frac{2}{p}}$ . In this expression  $p$  will be  $n - r - 1$ . In the application to the Broadbalk wheat yield  $n = 67$ ,  $r = 5$ , so the standard percentage error

$$100\sqrt{\frac{2}{p}} = 18.11.$$

The combined significance of a group of terms may be derived from Elderton's tables of goodness of fit, taking for  $\chi^2$  the ratio of the observed variance to  $v$ , and for  $n'$  one or more than the number of terms.

#### 9. *Magnitude of the Residuals.*

The coefficients of the fitting polynomial are given by the equations

$$A_p = \frac{S(T_p^x)}{S(T_p^2)},$$

where  $S$  represents summation over the  $n$  observations. The polynomial is therefore

$$\sum_0^r \frac{S(T_p^x)}{S(T_p^2)} T_r,$$

and the residual is

$$x \left\{ 1 - \sum_0^r \frac{T_p^2}{S(T_p^2)} \right\} - S' \left\{ \sum_0^r \frac{T_p T_p' x'}{S(T_p^2)} \right\},$$

in which  $S'$  represents summation over all observations except  $x$ .

If  $x$  and  $x'$  are independently distributed about a mean at zero then for a fixed value of  $t$  the mean square residual is

$$\sigma^2 \left\{ 1 - \sum_0^r \frac{T_p^2}{S(T_p^2)} \right\}.$$

$$\begin{aligned} \text{Now} \quad T_0 &= 1, & S(T_0^2) &= n, \\ T_1 &= t, & S(T_1^2) &= \frac{1}{2}n(n^2 - 1), \\ T_2 &= t^2 - n_2, & S(T_2^2) &= \frac{1}{10}n(n^2 - 1)(n^2 - 4), \end{aligned}$$

and so on.

The extreme value for  $t$  is  $(n - 1)/2$ ; for this value,

$$\begin{aligned} \frac{T_0^2}{S(T_0^2)} &= \frac{1}{n}, \\ \frac{T_1^2}{S(T_1^2)} &= \frac{3(n - 1)}{n(n + 1)}, \\ \frac{T_2^2}{S(T_2^2)} &= \frac{5(n - 1)(n - 2)}{n(n + 1)(n + 2)}, \end{aligned}$$

and so on.

The average value of the mean square residual for all values of  $t$  is

$$\sigma^2 \left( 1 - \frac{r+1}{n} \right),$$

as is necessarily the case since each term used removes  $\sigma^2/n$  of the variance; the reduction of variance is not the same for all values of  $t$ , and this introduces an element of heterogeneity. The reduction is greatest at the extremes. The polynomials tend to fit the extreme terms more accurately than the others. This effect is only strongly felt in the first and last terms. Fitting 67 terms with a curve of the 5th degree the variance of the first and last residual is reduced to 63.87 per cent. of its average value.

The residual variance for each value of  $t$  of a series of 67 terms, fitted to the 5th degree, is shown in the following table; the original variance being taken as 66, and the mean residue therefore 61.

Table VII.

$\pm t$		$\pm t$	
0	63.48	17	62.96
1	63.46	18	62.82
2	63.40	19	62.59
3	63.29	20	62.27
4	63.18	21	61.90
5	63.04	22	61.50
6	62.91	23	61.12
7	62.80	24	60.83
8	62.72	25	60.67
9	62.67	26	60.66
10	62.67	27	60.75
11	62.71	28	60.81
12	62.78	29	60.58
13	62.87	30	59.33
14	62.95	31	56.36
15	63.01	32	50.23
16	63.02	33	38.96

These are shown in Fig. 2.

The introduction of slight heterogeneity is a necessary consequence of the elimination of change by curve fitting; it is a weakness of the polynomial form that the extreme terms should be so much affected. The form of fitting polynomial is therefore unduly affected at its extremes by fortuitous circumstances; its values at the extremes give a less reliable index of mean yield than in the remainder of the range; still less is the rate of change at the extremes to be relied upon as a basis for prediction.

It should be noticed that for each year of the series separately the polynomial value is an approximation to the true mean for that year. The variance of the polynomial value about the true mean is complementary to that of the observation about the polynomial value (the



residual variance). Together they make up  $\sigma^2$ , the variance of the observation about its true mean. Thus the residual variance of the end values being only 38.96 parts in 67, the variance of the polynomial value must be 28.04; this is four times the average value so that the probable error of the extreme values of the polynomial is double as great as the average value for the whole series.

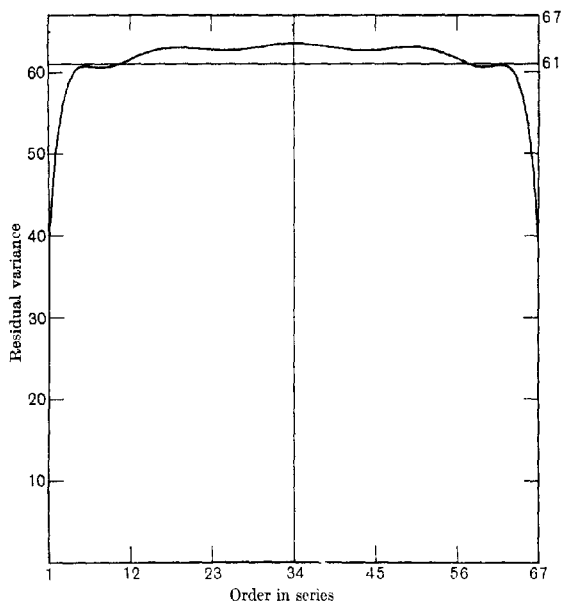


Fig. 2. Residual variance of individual terms, according to their position in the series.

#### 10. *Correlation of residuals.*

The formulae of the last section show that once the changes of the mean have been sufficiently represented, the addition of further terms to the polynomial is disadvantageous (i) in increasing the probable error of all values, (ii) in increasing the heterogeneity of the residuals. On the other hand, if an insufficient number of terms are taken (i) the residual variance will be overestimated, (ii) the residuals will be heterogeneous by the confusion of changes with annual variation.

It is possible to test whether the process of fitting has been carried far enough by means of the correlations between neighbouring

values. The correlation between any two deviations from the mean of  $n$ , is, if they are independent,

$$-\frac{1}{n-1};$$

while the correlation between neighbouring values of a changing series will generally be positive. For example, in the yields of dressed grain from the dunged plot, 2b, of Broadbalk, neighbouring values are evidently associated. The correlations between harvests 1, 2, 3, up to 6 years apart are:

	$r$	$z$	P.E.
1	+·3559	+·3722	±·0850
2	+·1637	+·1662	±·0857
3	+·2767	+·2841	±·0864
4	+·1811	+·1831	±·0871
5	+·2030	+·2058	±·0878
6	+·1488	+·1499	±·0886
Mean	...	+·2429	±·0354
Expectation	...	-·0152	—
Difference	...	+·2581	±·0354

(In calculating averages or differences of correlations, it is advisable to use, as above the function  $z$ , connected to  $r$  by the equation

$$r = \tanh z;$$

when  $r$  is small, as in these cases,  $z$  is little different from  $r$ ; for large values, however, the inaccuracies involved in using the probable error of  $r$  as though errors in  $r$  were normally distributed, become considerable.)

Evidently neighbouring values tend to be alike. This correlation between neighbouring values gradually disappears as the changes in mean value are more and more closely represented by the polynomial, at the same time the negative correlation to be expected from an unchanging series is gradually increased. When these two values are no longer significantly different, the subsequent terms of the polynomial cannot be of importance.

The calculation of the correlation to be expected between residuals of  $k$  years apart, for an unchanging series or one in which the change is fully represented by the polynomial, is somewhat complex. When  $k$  is small compared to  $n$  it will be sufficient, as in the present applications of the theory, to ignore terms involving

$$\frac{k}{n-r-1}$$

to the third and higher powers. In these cases the correlation between residuals of the polynomial of degree  $r$  taken  $k$  years apart is

$$-\frac{r+1}{n-r-1} \left( 1 - \frac{rk}{n-r-1} - \frac{rk^2}{(n-r-1)^2} \dots \right).$$

Thus, after fitting the polynomial for 67 values up to the fifth degree, the mean correlations between residuals are:

<i>k</i>	<i>r</i>	<i>z</i>	P.E.
1	-.0902	-.0904	±.0850
2	-.0817	-.0819	±.0857
3	-.0730	-.0731	±.0864
4	-.0640	-.0641	±.0871
5	-.0547	-.0547	±.0878
6	-.0452	-.0452	±.0886
Mean	...	-.0687	±.0354

The figures for plot 2*b* are even more negative than the expectation.

	<i>r</i>	<i>z</i>
1	+.0034	+.0034
2	-.1682	-.1698
3	-.0624	-.0625
4	-.1490	-.1501
5	-.0583	-.0584
6	-.0407	-.0407
Mean	...	-.0797
Expectation		-.0687 ±.0354
Difference		-.0110 ±.0354

Neighbouring residuals of this series are not less unlike than would be expected from an unchanging series; it may be inferred that the polynomial of the fifth degree sufficiently represents the course of the slow changes.

### III. POSSIBLE CAUSE OF SLOW CHANGES.

#### 11. *The local character of the slow changes.*

The distinction between the slow changes in mean yield and the annual variations is emphasised by the extremely local character of the former. An examination of the successive yields (*a*) of the experimental wheat at Woburn, (*b*) of the wheat averages for Hertfordshire compiled by the Board of Agriculture, (*c*) of barley, and (*d*) of grass from the experimental plots at Rothamsted, shows that the slow changes of mean yield observed on Broadbalk are not reflected in these series. To make the test objective correlation coefficients were found between the yields on plot 3*a* of Stackyard Field, Woburn, and (1) the actual yields of plot 2*b* of Broadbalk, (2) the polynomial values and (3) the deviations from the polynomial for the same plot.

Table VIII.

Correlations of plot 3*a* of Stackyard  
Field, Woburn, with

	<i>r</i>	<i>z</i>
Actual yield ...	+.31	+.32 ±.12
Polynomial value	+.02	+.02 ±.12
Deviations ...	+.34	+.35 ±.12

There is no significant association of the polynomial value with the yields on Stackyard Field, Woburn. The latter values are indeed more closely associated with the deviations from the polynomial than with the actual yields. The correlations are all somewhat low, perhaps owing to the very different soil at Woburn. The probable errors refer to deviation in the value of the correlation to be expected in a random sample of 36 years, the three values above are, however, taken for identical years, so that the probable error gives no indication of the significance of their differences.

Table IX.  
Correlation of Hertfordshire mean  
wheat yield with

	$r$	$z$
Actual yield ...	+·31	+·32 $\pm$ ·13
Polynomial value	-·16	-·16 $\pm$ ·13
Deviation ...	+·55	+·61 $\pm$ ·13

As before, the correlation with the polynomial value is not significant. Its actual value is negative, and the effect of including the polynomial terms in the actual yield, is to reduce the strong positive correlation shown by the deviations to a value which by itself would not be clear proof of any association whatever.

The comparison with the dunged barley plot on Hoos Field is interesting, since it might be suspected that the slow changes in mean yield, at any rate on the dunged plots, were partially caused by changes in the manurial value of the farmyard manure employed. That this effect is not of great importance may be seen by comparing the polynomials from the dunged plot with that of plots receiving only artificial manures (Fig. 3). This conclusion is confirmed by the comparison with Hoos barley.

Table X.  
Correlation of plot 7 (2) of Hoos  
Barley Field with

	$r$	$z$
Actual yield ...	+·28	+·29 $\pm$ ·09
Polynomial value	+·09	+·09 $\pm$ ·09
Deviation ...	+·23	+·23 $\pm$ ·09

All the correlations are small, and that with the polynomial is insignificant. The inclusion of these latter has in this case slightly increased the correlation observed from the actual yield.

With the yield of the grass plots the correlations are negative, but are all insignificant.

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Table XI.  
Correlation of plot 9 (first crop) of  
Park Grass with

	$r$	$z$
Actual yield ...	-.19	$-.20 \pm .12$
Polynomial value ...	-.12	$-.12 \pm .12$
Residuals ...	-.12	$-.12 \pm .12$

Thus the correlations of the residual yields with neighbouring wheat crops is clearly significant and greater than that of the actual yields, with barley and hay the correlations are less distinct; in no case do the polynomial values give significant correlations. The causes of the changes in mean wheat yield on Broadbalk do not affect the wheat yields of Woburn or Hertfordshire, or the barley and hay of neighbouring fields at Rothamsted; they appear to be limited to Broadbalk field.

The relation between yield and weather cannot be fully treated here; indeed, the present results may be regarded as preliminary to a thorough attack upon that problem. It is here only necessary to justify the assumption that the weather of a succession of years may be treated as an unchanging series (see Section 7). A full investigation of this point cannot be attempted until the whole meteorological data from the Rothamsted Station has been analysed. As an example of the method of investigation appropriate for testing this question in its present application we may cite the case of October rain.

Hooker (1907) found that the rainfall in autumn was more influential than that at other times of the year. The series of October rains was therefore examined to see if any change in mean rainfall could be detected. To do this accurately the series was fitted with a polynomial of the fifth degree, exactly as had been done to the wheat yields, and the chance of obtaining larger coefficients from an unchanging series was calculated. Every term individually proved to be insignificant, the chance of finding greater variance in the first 5 terms of an unchanging series being .88 (compare Table II). There is thus no evidence of more than random changes in the October rain.

One significant, though small, correlation was found between the polynomial values and the weather. On examining the correlation of total rainfall for each harvest year (Sept. to Aug.) with the succeeding crop, the following values were found.

Table XII.  
Correlation of total rainfall with

	$r$	$z$
Actual yield ...	-.60	$-.69 \pm .09$
Polynomial value ...	-.29	$-.29 \pm .09$
Deviations ...	-.56	$-.64 \pm .09$

This would indicate that a perceptible proportion, nearly 9 per cent. of the variance of the *mean* yield is due to difference in the year's rainfall. A further point of interest in connection with this result is that the optimum rainfall derived from the deviations is about 21 inches, that from the actual yields 17 inches, while for the polynomial value there is no optimum at all. This suggests that ideal conditions for the wheat plant on this soil requires a rainfall about 21<sup>1</sup> inches, but that lower values though somewhat injurious to the plant, are of permanent value to the field by facilitating the eradication of weeds.

If this be so, it illustrates a point to be borne in mind in considering the effect of weather upon crops, that the ideal weather for the plant is not necessarily ideal for the purpose of farm operations; it is not to be expected that in every case these two classes will be distinguishable as above, the one as an annual effect, the other with relatively permanent consequences.

#### 12. *Possible influence of weeds.*

Of all the organic factors which influence the yield of wheat, it is probable that weeds alone change sufficiently slowly to explain the changes observed on Broadbalk. Farm weeds are notoriously difficult to eradicate; deep-rooted perennials throw up stems year after year from below the cultivated layer, whilst annuals, and on Broadbalk especially the slender Foxtail grass, produce numerous seeds which may lie dormant for several years, so baffling the most thorough attempts to free the field once it is badly infested. The conditions of cultivation of Broadbalk make the control of weeds peculiarly difficult. The wheat, grown year after year, has been sown in autumn in every year save one (1853) leaving but a short space of time for cleaning the land. The mean date of autumn sowing is Nov. 1.4 and that of carting the crop is Aug. 24.9, leaving a mean interval of only 68.5 days. Compared to fields bearing spring-sown corn, or periodical root crops the position of Broadbalk is exceedingly unfavourable. It is therefore not improbable that if for a period the weeds are unchecked either from a relaxation of effort, or from unfavourable seasons, a considerable number of successive wheat yields would be reduced, giving rise to such periods of depressed yield as have been observed in the early fifties, and the seventies of the last century and about 1914.

<sup>1</sup> This refers to rainfall as *actually distributed* throughout the year. To discover the optimum distribution of rainfall will require a far more elaborate analysis.

## 13. Records of Weeds.

(1) An old record exists giving the exact work of every man employed in each day. The record includes parts of the four years 1852 to 1855, and is complete for the year 1853. During this period the wheat yields show that the condition of the field was improving even on the unmanured plots. The amount of hand labour employed on Broadbalk is striking; in 1853 211.5 man-days and 714 boy-days were expended in weeding the field. The whole period of weeding operations little exceeds 100 working days, so that roughly the work done was equivalent to the continuous labour of two men and seven boys, on a field of about 14 acres. The proportion of boy labour is striking and the record is sufficiently detailed to show how it was utilised.

The weeding done by the boys was principally by hand, with some spudding in May or June. Only men used hoes. In April boys were at work "picking twitch," in June they were "pulling garlic" (presumably charlock) "and the larger weeds," and in July they were "pulling wild oats" in the high corn.

If our interpretation of the change in mean yield is correct, this free use of boy labour was eminently successful, for the mean yield mounts rapidly to its maximum about 1860. Unfortunately, no comparable record of the employment of labour exists at other dates.

(2) In the Broadbalk records the first botanical account of weeds is in 1867, a date corresponding to the beginning of the second depression in the mean yields (see Fig. 1). From then to 1889 the field is frequently described as "exceedingly foul"; from 1889 the weeds are not mentioned till 1904, when the weeds were sufficiently dominant to require that the field should be fallowed, as was done by halves in 1904-5, and again in 1914-15. The evidence of the records thus confirms the supposition that the field suffered from weeds at periods about 1877, and 1910, and was relatively free in the high-yielding period in the nineties.

From the observations of the seventies it is clear that the dominant weeds consisted of five perennials, *Sonchus arvensis* (Corn Sowthistle), *Convolvulus arvensis* (Bindweed), *Equisetum arvense* (Horsetail), *Cirsium arvense* (Creeping Thistle), and *Agrostis vulgaris* (Twitch grass), and three annuals, *Polygonum aviculare* (Knotgrass), *Myosotis*<sup>1</sup> *arvensis*

<sup>1</sup> In the opinion of Dr Brenchley, whom I have had the privilege of consulting, it is to be doubted that this weed, even when conspicuous, was really present in sufficient quantity sensibly to depress the yield.

(Birdseye), and *Stellaria*<sup>1</sup> *media* (Chickweed). A fourth annual, the Slender Fox-Tail grass, *Alopecurus agrestis*, was certainly regarded as an unimportant weed in 1867 and 1869, and is not mentioned in 1872, 1873 and 1876, but in 1879 and the eighties it has become enormously abundant, and at the present time it is considered to be by far the most troublesome weed. In 1886 this weed had become such a pest that "Sir John decided that pulling up by hand should be resorted to." In connection with this, it may be remembered that the Education Acts of 1876 and 1880 made attendance at school compulsory; the boy labour which had regularly hand-weeded the land in the past had evidently been cut off for some time before 1886. Great efforts were made again in 1887 to eradicate the weed by hand weeding, but the wet summers of 1888 and 1889 prevented this operation and the land again became very foul, and was partially fallowed in 1890 and 1891 by drilling the rows at double widths over half the field. After this the weeds seem to have been kept in check until Sir John Lawes' death in 1901<sup>2</sup>. In 1904 *Alopecurus agrestis* was so thick that the field was given a complete fallow in two halves in 1904, 1905.

#### 14. *Indirect evidence of the influence of weeds.*

Much evidence has already been adduced that the slow changes in the mean yield is due to very different causes from those that produce the annual deviations; in particular, the local character of the former has been mentioned. The variation due to those two causes, *relative to the mean of each plot*, may be conveniently expressed as a coefficient of variation.

The most striking point about these figures is the comparative constancy of the coefficient of variation for slow changes, especially for neighbouring plots, if we exclude plots (17 and 18) for separate discussion. The variation is uniformly greater in the north half (plots 2b to 8) of the field than in the more southern portions (plots 10 to 14), the localisation of the effect thus showing itself even within a single field. The coefficients of variation from annual causes are quite different; they show no influence of locality, the most variable being those plots in which exhaustion has been most rapidly in progress. The proportionality

<sup>1</sup> Possibly *Arenaria serpyllifolia* (Sandwort) was sometimes recorded as *Stellaria media*; the latter weed is, however, the more frequent in recent reports.

<sup>2</sup> During the nineties parties of schoolgirls were employed at hand-picking, at Easter and Whitsun, on Saturdays and in the evenings. Sir John Lawes took much interest in their work, giving prizes to those who collected the greatest quantity.



of the slow changes is well shown by plotting the polynomials upon a logarithmic scale, as in Fig. 1, which shows the changes in mean yield on plots 2b to 14.

Table XIII.

Coefficient of variation due to slow changes and annual deviations

Plot	Slow changes	Annual deviation
2b	12.13	16.68
3 and 4	12.86	24.90
5	11.56	24.17
6	12.53	24.51
7	11.61	22.61
8	11.16	20.44
10	9.83	33.32
11	9.33	32.13
12	9.88	26.98
13	9.54	23.56
14	9.00	25.26
17 } minerals	14.72	31.88
18 } ammonia	10.35	21.32

This proportionality, while discriminating the slow changes sharply from the meteorological effects, is not an unexpected feature if these changes are due to the varying prevalence of weeds. For in a bad period perhaps 25 per cent. of the area of each plot is unproductive, while after many years of constant attention the percentage lost may be reduced to about 5 per cent.; the constancy of the coefficient of variation in the different plots indicates that the area lost in the same year is roughly proportional in all plots.

An exceptional amount of variation is shown by the mineral series of plots 17 and 18; this plot differs from the others in that while the growth of wheat is limited by shortage of nitrogen, in the previous year it received a nitrogenous dressing. This should be to the advantage of the perennial weeds which should have benefited by the previous year's manure. The mineral plot should therefore suffer particularly when the field is infested by perennial weeds, while the corresponding ammonia plot should show principally the effect of annual weeds.

A comparison of the polynomial for plot 17 and 18 minerals with that for plot 5 which it nearly resembles, shows that (Fig. 3) the former was in fact more seriously depressed in the first two periods of low yields, to such an extent that it has a lower average in the early fifties and about 1880, while in the recent depression the effect is less marked, its minimum being higher than that of plot 5. On the other hand, comparing plot 17 and 18 ammonia with plot 7, which receives

ammonia continuously, the first two depressions of the former are considerably less, while the recent depression is quite as great as that of the latter. These differences are comprehensible if in the two earlier

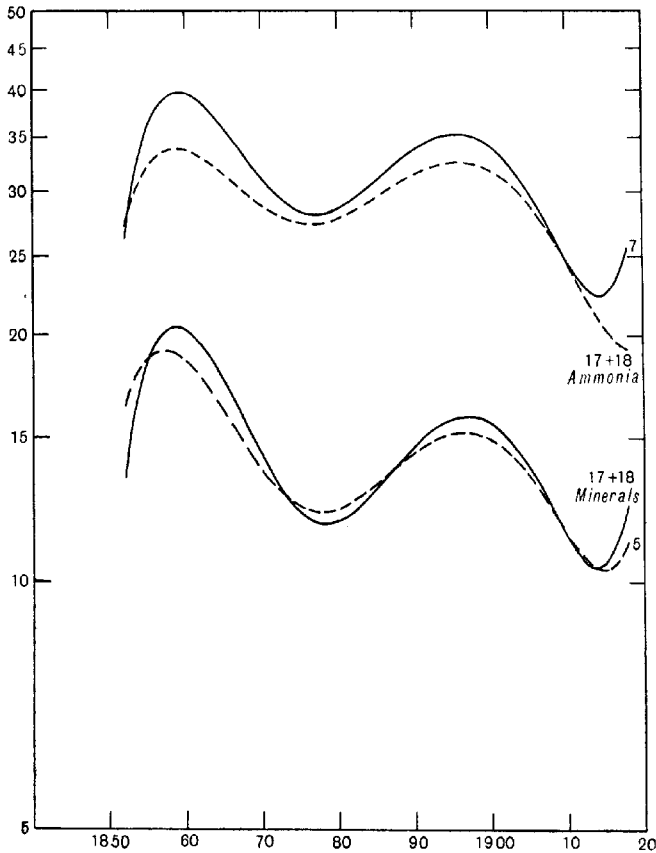


Fig. 3. Yield of alternating plots 17 and 18, compared with plots 5 and 7; showing the exaggeration of the two earlier depressions, where a nitrogenous dressing has been applied in the previous year.

depressions perennial weeds were more influential than has been the case in recent years, while the recent depression is due in larger measure to annual weeds. Similar conclusions have been seen to be indicated by the references to weeds in the records.

## SUMMARY.

In Part I is given a survey of the results of a statistical examination of the yield of the plots of Broadbalk Wheat field during 67 years. The main features of the comparison of mean yields are well known; the comparative rates of decrement, shown in Section 5, supply a class of facts well worthy of further study. Particularly striking are the relatively slow rates of decrement of plots 2*b* and 8, compared with plot 7, which would seem to show a permanent advantage in very high nitrogenous dressings, and to emphasise the need for caution in the application of the principle of diminishing returns. The evidence of the influence of potassium sulphate and its substitutes, sodium sulphate and magnesium sulphate, shown in Table V, is also very striking. An unsuspected feature of the changes of mean yields, which precludes the possibility of obtaining from these data true curves of exhaustion has appeared in the slow changes which have taken place in all the plots in a similar manner. In Part II the mathematical methods by which the variation has been analysed has been discussed, partly as a justification of novel procedure, partly to make clear that the three types of variations found have been genuinely distinguished. In Part III such evidence as is available has been presented, in order to throw light upon the possibility that the changes in mean yield have been caused by variations in the prevalence of weeds at different periods.

One point of importance which should be emphasised is that average wheat yields, even over long periods, from different fields or for different seasons cannot approach in accuracy the comparison of plots of the same field in the same seasons. The advantage of the method adopted by Lawes in the permanent experiments which he instituted is very evident. The effects of weather clearly require that the seasons should be identical, unless the series be very long, but the slow changes in mean yield show that even comparatively long series of different years from the same field cannot be accurately compared. Within the same field, however, the slow changes have almost proportional effects, and comparison between the mean yields of neighbouring plots may be made with great accuracy. The only case in which changes in mean yield sensibly affect the comparison of averages is that of plots 17 and 18. In comparing these with plots 3 and 4, 5, 7, and 10, it would be more accurate to confine attention to high yielding periods, at which the disturbing causes are at their minimum.

It is believed that the deviations from the smooth curves, which have been freed, for the most part, from the effects of exhaustion and weeds, form statistically homogeneous material for the study of meteorological effects.

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## DEPHENOLISATION IN SOIL.

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(With Six Figures in Text.)

### INTRODUCTION.

THE antiseptic property of the phenols is so well known that the idea of their bacterial decomposition seems a paradox. Yet, when the fundamental cycle of changes occurring in the organic world is considered, the decomposition of the phenols, biological or otherwise, would seem almost inevitable. Phenol and *p*-cresol are formed in the intestine by the bacterial decomposition of proteins containing tyrosine and are finally excreted in urine, to find their way, sooner or later, largely into the soil. Liechti and Mooser<sup>1</sup> estimated that the amount of the phenols applied to the land in liquid manure would be anything between 34 and 83 kilos per hectare<sup>2</sup> (presumably per dressing). The production of phenols from tyrosine is probably not confined to the intestine; it might occur in other decompositions of proteins containing tyrosine. That being so, the ultimate fate of these compounds is of considerable significance in the economy of nature.

Some work on the subject has already been done. Fowler, Ardern and Lockett<sup>3</sup> showed that phenol is oxidisable by a chromogenic bacillus which they isolated from sewage filters. Mooser<sup>4</sup> studied the disappearance of phenol and *p*-cresol in the soil which he attributed to enzyme action in spite of fruitless efforts to isolate an appropriate enzyme. Buddin<sup>5</sup> at Rothamsted observed that doses of phenol, not exceeding 0.1 per cent., induced a temporary rise in the numbers of certain

<sup>1</sup> *Landw. Jahrb. Schweiz.* 1906, 1.

<sup>2</sup> Roughly, anything between 30 and 74 lb. per acre.

<sup>3</sup> Fowler, Ardern and Lockett, "The oxidation of phenol by certain bacteria in pure culture," *Proc. Roy. Soc.*, 1911, B 85.

<sup>4</sup> W. Mooser, "Biologisch-Chemische Vorgänge im Erdboden," *Landw. Vers. Stat.* 1911, 75.

<sup>5</sup> W. Buddin, "Partial sterilization of soil by Volatile and non-Volatile antiseptics," *Jour. Agri. Sci.* 1914, 6.

types of bacteria in the soil, which he attributed to a feeding effect of phenol. Almost contemporaneously with the publication of Buddin's paper, Wagner<sup>1</sup> in Germany showed the wide distribution in nature of organisms capable of decomposing phenol and many other aromatic compounds, including benzene, toluene, xylenes, catechol, phloroglucinol, and utilising their carbon as a source of energy. He isolated no fewer than seven of these organisms from various sources—the atmosphere, soil and animal excreta; he studied the changes, however, in culture solutions, but not in nature.

The writer's investigations show that phenol and the cresols are rapidly attacked in the soil and that their disappearance is brought about by several agencies of which one—apparently purely chemical or physico-chemical—acts instantaneously and the others more slowly. The slow-acting agencies are partly biological and partly chemical. These agencies will now be discussed in detail.

## PART I.

### THE SLOW DEPHENOLISATION.

The curves for soils receiving farmyard manure suggest bacterial action, though with fresh soil some of them are not entirely typical. More typically bacterial curves, however, are obtained when soil is partially sterilised, before the application of phenol, by treatment with toluene which is then evaporated, and also by drying for a day and then re-moistening. The disappearance is by no means stopped by leaving toluene in the soil during the whole period of the experiment, but the action is considerably retarded. The effect of treating the soil with mercuric chloride has been so similar to that of toluene left in that the two curves are almost superimposable. These results indicate the possibility of a purely chemical reaction between phenol and some substance which is capable of being produced at a slow rate in the soil, this reaction proceeding simultaneously with the bacterial decomposition.

On the other hand, autoclaving the soil at 130° C. for 20 minutes stops the action altogether.

The rates of the disappearance of phenol are of much the same order in all the soils tested receiving farmyard manure; but there is a fundamental difference between the rate in manured and that in unmanured soil. The curve for the latter is practically a straight line and it seems

<sup>1</sup> R. Wagner-Basel, "Gärung, Fäulnis und Verwesung," *Zeits für Gärungsphysiologie*, 1914, B. 4, H. 4.

very probable that the loss in the unmanured soil is *mainly* due to the action of the agency which operates in presence of toluene and mercuric chloride.

At least two types of bacteria have been met with in various soils and in stable manure, which readily break down phenol in culture solution containing carbon in the form of phenol only. They have been isolated in a pure state for future detailed study.

A bacillus which decomposes *m*-cresol in culture solutions has also been isolated from the soil. These are being further studied in the bacteriological laboratory.

#### RECOVERY OF PHENOL FROM SOIL AND ITS ESTIMATION.

Although an hour's steam-distillation (yielding 500 c.c. of distillate) is quite sufficient for the complete recovery from an aqueous solution of the largest quantity of phenol used in the present investigation (namely 0.05 gm.), experience shows that much more prolonged distillation is necessary for maximum recovery from soils. Except where very small amounts are to be recovered the first hour's distillate contains as much as 96 to 98 per cent. of the recoverable phenol: the rest, for reasons not clearly understood, being recovered only on prolonged distillation. Consequently the soil is distilled for at least  $5\frac{1}{2}$  hours, no matter what its phenol content. The distillate is collected in three lots of 500 c.c. each, occupying 1, 2, and  $2\frac{1}{2}$  hours respectively.

For the estimation of phenol in the distillate the well-known bromination method proved unsuitable. Two colorimetric methods were therefore used. A red colour is produced when phenol is heated with small quantities of Millon's reagent and strong nitric acid and under strictly standard conditions the intensity of colour for a given amount of phenol is constant. The method can be made to yield satisfactory results (of 98 per cent. accuracy with the higher standards) for any amount down to 0.25 mgm. in 35 c.c. of the distillate, but below this and down to 0.05 mgm. the colour produced is so feeble as to be useful only for qualitative purposes. For these smaller quantities the second colorimetric method was used, which consists in nitrating phenol in the cold with sulphuric and nitric acids, and, after standing, neutralising with ammonia. The yellow colour produced can be used for the estimation of as small an amount of phenol as 0.01 mgm. in 50 c.c. of the distillate. The accuracy of this method is, however, of a much lower order than that of the Millon's reagent method, but in a large majority

of cases it has been used only for the estimation of a very small fraction of the total amount of phenol.

Both methods work equally well for the cresols, but the conditions that produce the best colour with Millon's reagent differ both among themselves and also from those in the case of phenol.

#### METHOD OF INVESTIGATION.

The soil, freshly taken from the top four inches of the field, was well mixed; stones, worms and undecomposed vegetable remains being picked out; it was then passed through a 3 mm. sieve (except when too sticky), weighed out in lots of 100 gms. each and placed in round-bottomed litre flasks in which the soil was subsequently distilled, and treated with an aqueous solution of phenol. In the early experiments 2 c.c. of water containing 50 mgms. of phenol were sprayed on to the soil as completely as possible from a spraying-bottle, the soil being well shaken meanwhile. Then 2 c.c. of water were introduced, shaken gently inside the spraying-bottle and sprayed as before. The amount of phenol left in the bottle was practically constant, roughly 0.25 mgms., so that the amount of phenol sprayed on was taken to be 47.75 mgms. in each unit of an experiment. Later on, by suitable modifications as much as 49.8 mgms. out of 50 could be sprayed. In later experiments the requisite volume (always corresponding to 50 mgms.) of phenol solution was dropped from a pipette direct on to the soil, care being taken that not more than one drop fell on the same patch of soil, after which the soil was shaken so as to get a fairly uniform distribution. The flasks were loosely plugged with cotton-wool (except in the cases of a few special experiments) and put away on a shelf in a dark cellar until wanted for distillation. No effort has been made to maintain rigidly constant temperature; but the records show a variation of only 0.5° about a mean of 16° C.

*The soils used were as follows:*

1. Barnfield "dunged" soil. (Plot 1, mangold expts.)
2. Barnfield unmanured soil. (Plot 8, mangold expts.)
3. Dr E. J. Russell's garden soil.
4. Heavily manured cucumber-house soil.
5. West Barnfield soil.

The Barnfield "dunged" plot has received a dressing of 14 tons per acre of farmyard manure every year, in addition to the mangold leaves from the plot which are spread over the surface after the roots have been pulled: no artificial manures are given. The unmanured plot has received no manure whatever since 1856, except in the form of mangold



leaves. As would be expected, the manurial treatment has brought about a considerable difference in the physical texture of the two soils besides the obvious chemical differences, the dunged plot being lighter than the other, though both are heavy loams.

Dr Russell's garden soil is rich in organic matter, fairly light and capable of holding much moisture.

The cucumber-house soil is the richest soil used in these experiments, having received much stable manure, dried blood, fish guano and a special mixture of artificial manures for cucumbers; it has an exceptionally high moisture content. It was used in a cucumber-house in 1919 and lay in a large heap outside since September of that year.

The soil of West Barnfield is the lightest at Rothamsted, and was much used during the winter months when most of the other soils are too sticky in the fresh condition for satisfactory work. The field is cropped in rotation and received stable manure in the autumn of 1917 for the crop of potatoes in 1918, which also received artificial fertilisers. In the spring of 1919 a top dressing of sulphate of ammonia was applied.

#### RATES OF DISAPPEARANCE OF PHENOL IN DIFFERENT SOILS IN THE FRESH CONDITION.

In Barnfield "dunged" soil the loss was rapid: the results are given in Table I and plotted in Fig. 1.

Table I.

##### *Disappearance of Phenol in Barnfield "dunged" soil.*

Wt of soil—100 gms.; % of moisture in soil = 11.64.

Added moisture with phenol—4 c.c.

Wt of phenol *sprayed* on = 47.75 mgms.

Period of contact with soil	Mgms. phenol recovered	% phenol lost
20 minutes	40.7	14.76
1 day	24.99	47.66
2 days	17.84	62.64
3 „	12.51	73.80
4 „	1.55	96.75
5 „	1.09	97.73
6 „	0.85	98.22
7 „	0.85	98.22

In Barnfield unmanured soil the loss was much slower and continued for a period of 22 days. The results are given in Table II and plotted in Fig. 1.

Table II.

*Disappearance of Phenol in Barnfield unmanured soil.*

Wt of soil = 100 gms.; % of moisture in soil = 14.29.

Added moisture = 4 c.c.

Wt of phenol *sprayed* on = 49.8 mgms.

Period of contact with soil	Mgms. phenol recovered	% phenol lost
20 minutes	39.92	19.84
1 day	33.76	32.21
3 days	30.35	39.06
7 "	25.19	49.42
14 "	17.74	64.38
22 "	0.75	98.49

In Dr Russell's garden soil the loss was rapid: the results are shown in Table III and in Fig. 1.

Table III.

*Disappearance of Phenol in Dr Russell's garden soil.*

Wt of soil = 100 gms.; % of moisture in soil = 25.92.

Added moisture = 4 c.c.

Wt of phenol *sprayed* on = 49.8 mgms.

Period of contact with soil	Mgms. phenol recovered	% phenol lost
20 minutes	40.72	18.23
1 day	34.93	29.86
2 days	29.96	39.84
3 "	24.62	50.56
4 "	10.94	78.03
5 "	3.40	93.17
7 "	2.40	95.18
11 "	1.81	96.37
14 "	1.45	97.09

The cucumber-house soil presented some difficulty because, when subjected to steam-distillation by itself, it gives off, after the first hour and at a progressive rate, one or more volatile decomposition products which react like the phenols in giving rise to a brilliant yellow colour on nitration and subsequent neutralisation with ammonia. During the first hour there is very little of this change. In Table IV, therefore, the figures show the phenol recovered in the first hour's distillate, the results for the second and third lots of distillate being impossibly high. Since 96-98 per cent. of the added phenol is generally recovered in the first hour's distillate, the figures may safely be taken to indicate the rate of disappearance of phenol. The curve is shown in Fig. 1.

Table IV.

*Disappearance of Phenol in cucumber-house soil.*

Wt of soil = 100 gms.; % moisture = 52.44.

Added moisture = 2 c.c.

Wt of phenol dropped from a pipette = 50 mgms.

Period of contact with the soil	Mgms. phenol recovered in 1st hour's distillate	% phenol lost
20 minutes	43.75	12.50
1 day	40.00	20.00
2 days	18.18	63.64
3 "	2.10	95.80
4 "	0.74	98.53
6 "	0.50	99.00

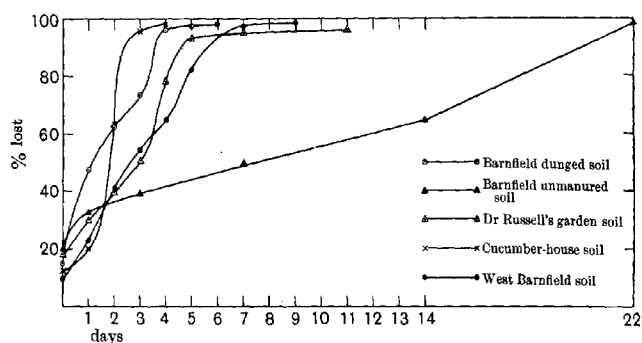


Fig. 1. Showing rate of disappearance of phenol in different soils.

Table V.

*Disappearance of Phenol in West Barnfield Soil.*

Wt of soil = 100 gms.; % moisture = 21.39.

Added moisture = 2 c.c.

Wt of phenol dropped from a pipette = 50 mgms.

Period of contact with the soil	Mgms. phenol recovered	% phenol lost
20 minutes	45.28	9.44
1 day	38.62	22.76
2 days	29.56	40.88
3 "	22.89	54.22
4 "	17.59	64.82
5 "	8.81	82.38
7 "	1.35	97.30
9 "	0.89	98.22

West Barnfield soil, being the lightest and easiest of the Rothamsted soils to handle during winter, was employed in all work on the effects of different conditions on the disappearance of phenol. The results given in Table V and Fig. 1 thus become the standard with which most of the subsequent data will be compared.

#### DISCUSSION OF THE FOREGOING RESULTS.

A striking point about the series of curves drawn in Fig. 1 is the widely different character of the curve for the unmanured soil from those of the others, which all resemble one another. After the first day, the curve for the unmanured soil is a straight line till the 14th day, after which there is a slight upward concavity.

The agent effecting the disappearance of phenol in the dunged soils, which the writer believes to be mainly biological, plays only a small part in the unmanured soil. The similarity of the curve for the unmanured soil to those for the disappearance of phenol in soil treated with toluene (left in) and mercuric chloride suggests that the principal agency is neither biological nor enzymic. It is not suggested, however, that the unmanured soil is altogether free from the phenol-splitting organisms. There is evidence of the presence in this soil of one of the two isolated types of organisms—a fact which may be accounted for by aerial infection. But the organisms must be present in very small numbers only, and exert little influence until towards the end of the disappearance period. The concavity of the curve between the 14th and 22nd days of incubation may be attributed to the bacterial action supplementing the main change which the writer suggests is determined by some substance or substances capable of being produced in the soil at a slow rate.

The other curves of the series are all strongly suggestive of bacterial decomposition, although some of them (more especially the curve for Barnfield "dunged" soil) present difficulties. The curve most typical of bacterial action is the one for rich cucumber-house soil. Here an initial slow action is followed by a very rapid action due to the enormous multiplication of the bacteria feeding upon phenol. Most of the curves show a certain "tailing off" of the activity towards the end of the disappearance period: this effect occurs also in many later experiments, but its precise cause is not clear. The phenomena observed during the disappearance of successive doses of phenol in the same soil indicate that this slackening of activity is not due to the accumulation of products of decomposition.

There may be two factors at work in Barnfield "dunged" soil: the one, mainly responsible for the first part of the loss, possibly loses its importance before the second becomes fully operative. In a later repetition a similar high rate of loss during the first day was observed, amounting to 38.56 per cent. as against 47.66 per cent. in the experiment under discussion.

#### DISAPPEARANCE OF SUCCESSIVE DOSES OF PHENOL.

This experiment emphasises the essentially bacterial character of the disappearance of phenol. Soil freshly obtained from West Barnfield was placed in lots of 100 gms. each in a number of round-bottomed

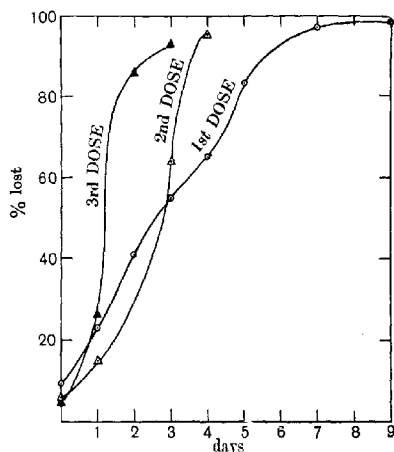


Fig. 2. Showing disappearance of successive doses of phenol in the same soil.

Table VI.

*Showing the disappearance of the second and third doses of Phenol.*

Wt of soil=100 gms. Wt of phenol=50 mgms.

Period of contact with soil	2nd dose		3rd dose	
	Mgms. phenol recovered	% phenol lost	Mgms. phenol recovered	% phenol lost
20 minutes	47.02	5.96	—	—
1 day	42.75	14.15	36.94	26.12
2 days	—	—	7.36	85.28
3 "	18.00	64.00	3.62	92.76
4 "	2.47	95.06	—	—

flasks and treated with phenol in the usual manner. The contents of one of the flasks were distilled after nine days' standing: 1.43 mgms. phenol was recovered out of the original 50 mgms. On the following day the contents of the remaining flasks were treated with a second dose of phenol (50 mgms. in each case) and estimations made after one, three and four days respectively, when practically the whole of the second dose was found to have disappeared. On the 15th day after the commencement of the experiment the contents of the remaining flasks were treated with 50 mgms. phenol each for the third time. The disappearance of the third dose was even more rapid than that of the second, 86 per cent. being lost in two days (Table VI, Fig. 2): the curve for the first dose has been taken from the experiment of Fig. 1; the other two belong to the present experiment.

#### EFFECT OF AUTOCLAVING AT 130° C. ON THE FATE OF PHENOL.

Five lots of 100 gms. each of West Barnfield soil were autoclaved in round-bottomed flasks at 130° C. for 20 minutes, the flasks being closely plugged with cotton-wool. 50 mgms. of phenol were then added in the usual manner to the soil in each flask. Estimations of phenol were made after 20 minutes, and again once a week for the next four weeks. The results are set out in Table VII. The reading taken on the 28th day indicates a loss of 9 per cent. which might well be the tardy result of slight aerial infection suffered at the time of the application of phenol after autoclaving.

Table VII.

#### *Effect of Autoclaving on the disappearance of Phenol.*

Wt of soil = 100 gms.		Wt of phenol = 50 mgms.	
Period of contact with soil	Mgms. phenol recovered	% phenol lost	
20 minutes	49.51	None	
7 days	49.2		
14 "	49.38		
21 "	48.69	2.62 (?)	
28 "	45.48	9.04	

#### EFFECT OF TOLUENE ON THE DISAPPEARANCE OF PHENOL.

##### A. *Toluene left in.*

West Barnfield soil was placed in lots of 100 gms. each in a number of round-bottomed flasks and treated with toluene (5 c.c. per 100 gms. soil) dropped from a pipette so as to secure fairly uniform distribution.

The flasks were stoppered with corks to prevent the escape of toluene, shaken and allowed to stand for three days before phenol was applied. They were stoppered again directly after the application of phenol.

It will be observed on reference to Table VIII and Fig. 3 that about 89 per cent. of the added phenol was lost in 19 days. Biological action is usually considered to be out of the question in presence of so much toluene; but the possibility of enzyme action still remains. It will be shown later that phenol disappears at much the same rate in soil heavily treated with mercuric chloride which would appear to prevent enzyme action. The simplest way of explaining these results is to attribute the disappearance of phenol to a reaction with some compound or compounds whose slow production in the soil is not much affected by the presence of either toluene or mercuric chloride.

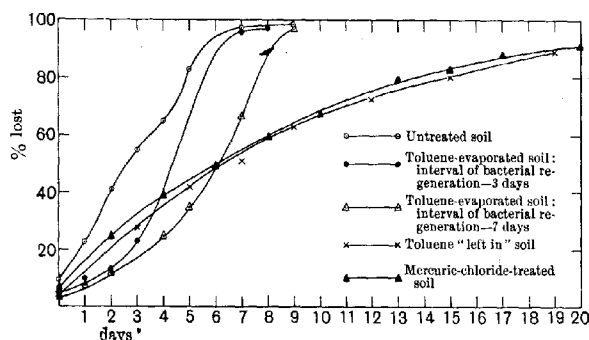


Fig. 3. Showing effects of various treatments on disappearance of phenol.

Table VIII.

*Effect of Toluene "left in" on the disappearance of Phenol.*

Wt of soil = 100 gms. Wt of phenol = 50 mgms.

Period of contact with soil	Mgms. phenol recovered	% phenol lost
20 minutes	47.85	4.30
3 days	36.26	27.48
5 "	29.01	41.89
7 "	24.41	51.18
9 "	18.36	63.28
12 "	13.85	72.30
15 "	9.95	80.10
19 "	5.60	88.80

B. *Toluene Evaporated.*

The experimental details were as follows:

A quantity of freshly obtained soil was treated in a large bottle with toluene at the rate of 5 c.c. per 100 gms., thoroughly shaken and left for two days, then spread out in a thin layer for a few hours to allow the toluene to evaporate, re-moistened and bottled for several days: then placed in lots of 100 gms. in round-bottomed flasks and treated with phenol in the usual way. Phenol estimations were made at suitable intervals.

The results of two experiments are set out in Table IX and Fig. 3. In the first the re-moistened soil was allowed to stand for three days before the application of phenol; in the second, it stood for seven days. The effect of the longer standing has been to lower slightly the rate of disappearance. Both curves suggest purely bacterial action: loss is slower during the first three or four days than in the untreated soil, but afterwards becomes very rapid.

Table IX.

*Effect of Toluene "evaporated" on the disappearance of Phenol.*

Wt of soil = 100 gms. Wt of phenol = 50 mgms.

Period of contact with soil	Interval before adding phenol—3 days		Interval before adding phenol—7 days	
	Mgms. phenol recovered	% phenol lost	Mgms. phenol recovered	% phenol lost
20 minutes	48.05	3.90	48.63	2.74
1 day	45.01	9.98	45.65	8.70
2 days	43.17	13.66	43.71	12.58
3 "	38.49	23.02	—	—
4 "	—	—	37.63	24.74
5 "	—	—	32.55	34.90
7 "	1.85	96.30	16.52	66.96
8 "	1.44	97.12	—	—
9 "	—	—	1.21	97.58

Another experiment was made to determine whether the effect could be increased by subjecting the same soil twice to the action of toluene with an intervening disappearance of phenol. The re-moistened soil, after three days' standing, was treated with phenol: four estimations were made in seven days, when practically all the phenol had disappeared. On the eighth day of the experiment the soils in the remaining flasks, in which presumably the phenol had disappeared at the same rate, were treated for a second time with toluene at the rate of 5 c.c. per 100 gms., left for two days, and then spread out in a thin layer for the toluene to evaporate



as quickly as possible. The soil was re-moistened and placed in round-bottomed flasks, again in lots of 100 gms. each, and treated with phenol immediately. The rate of disappearance of the second dose of phenol was considerably greater than that of the first, the change being almost complete in four days (Table X, Fig. 4). The results suggest that there is at least one spore-former among the phenol-splitting organisms.

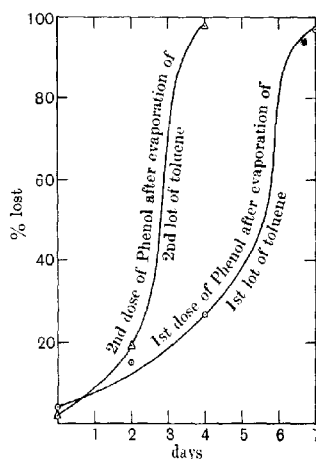


Fig. 4. Disappearance of successive doses of phenol in "Toluene-Evaporated" soil.

Table X.

Wt of soil = 100 gms. Wt of phenol = 50 mgms.

Period of contact with soil	First treatment		Second treatment	
	Mgms. phenol recovered	% phenol lost	Mgms. phenol recovered	% phenol lost
20 minutes	48.12	3.76	48.73	2.54
2 days	42.30	15.40	40.32	19.36
4 "	36.45	27.10	1.04	97.92
7 "	1.42	97.16	—	—

#### DISAPPEARANCE OF PHENOL IN SOIL HEAVILY TREATED WITH MERCURIC CHLORIDE.

A quantity of West Barnfield soil was air-dried for a day to 5.68 per cent. moisture, ground and passed through a 3 mm. sieve and thoroughly mixed with a 1 per cent. solution of mercuric chloride at the rate of 10 c.c. of the solution to 100 gms. of the air-dry soil. The moistened

soil was bottled immediately and left for three days, then lots of 100 gms. each were placed in round-bottomed flasks and phenol applied as usual. The flasks were loosely plugged with cotton-wool and put away in the cellar. Phenol estimations were made at intervals for a period of 20 days, when 90.4 per cent. of the added phenol had disappeared (Table XI, Fig. 3). A control experiment was made in which the above amounts of phenol and mercuric chloride were mixed in solution and left for 20 days: 92.74 per cent. of the phenol was recovered.

This undoubted disappearance of phenol in soil heavily treated with mercuric chloride suggests that soil can slowly generate some substance or substances which react with phenol.

Table XI.

*Disappearance of Phenol in soils treated with Mercuric Chloride.*

Wt of soil = 100 gms. Wt of phenol = 50 mgms.		
Period of contact with soil	Mgms. phenol recovered	% phenol lost
20 minutes	46.74	6.52
2 days	37.37	25.26
4 "	30.37	39.26
6 "	25.24	49.52
8 "	20.23	59.44
10 "	16.28	67.44
13 "	10.48	79.04
15 "	8.79	82.42
17 "	5.61	88.78
20 "	4.8	90.4

#### EFFECT OF STEAMING ON THE DISAPPEARANCE OF PHENOL.

Round-bottomed flasks containing 100 gms. West Barnfield soil each and closely plugged with cotton-wool were heated for an hour in a steamer. After cooling, the soil was treated as usual with phenol, and

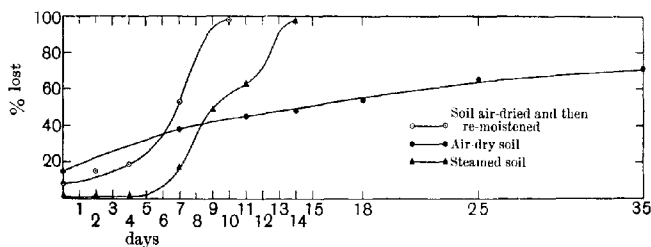


Fig. 5. Effects of air-drying, air-drying and re-moistening, and steaming on disappearance of phenol.

periodical estimations made during 14 days, when all the phenol disappeared (Table XII, Fig. 5). No loss was found during the first four days, then it proceeded very slowly until the seventh day; after which it was greatly accelerated. The results suggest that steaming affects bacterial activity differently from toluene. It is not clear to what this difference might be due.

Table XII.

*Effect of steaming on the disappearance of phenol.*

Wt of soil = 100 gms. Wt of phenol = 50 mgms.		
Period of contact with soil	Mgms. phenol recovered	% phenol lost
20 minutes	47.57	None
2 days	49.71	
4 "	49.34	
7 "	41.38	17.24
9 "	25.77	48.46
11 "	18.90	62.20
14 "	0.80	98.40

EFFECT OF AIR-DRYING AND OF SUBSEQUENT RE-MOISTENING  
ON THE DISAPPEARANCE OF PHENOL.

A quantity of West Barnfield soil was air-dried for a day to 2.4 per cent. moisture, ground and passed through a 3 mm. sieve; part was immediately re-moistened to 17.36 per cent. moisture. Each portion was divided into lots of 100 gms. in flasks and treated with phenol as before.

Table XIII.

*Disappearance of Phenol in air-dried soil and in soil air-dried and then re-moistened.*

Wt of soil = 100 gms. Wt of phenol = 50 mgms.

Period of contact with soil	Air-dried only		Air-dried and then re-moistened	
	Mgms. phenol recovered	% phenol lost	Mgms. phenol recovered	% phenol lost
20 minutes	42.49	15.02	46.12	7.76
2 days	—	—	42.43	15.14
4 "	—	—	40.67	18.66
7 "	30.80	38.40	23.38	53.24
10 "	—	—	0.83	98.34
11 "	27.50	45.00	—	—
14 "	25.66	48.66	—	—
18 "	22.98	54.04	—	—
25 "	17.63	64.74	—	—
35 "	14.77	70.46	—	—

In the re-moistened soil (Table XIII, Fig. 5) 98 per cent. of the phenol had disappeared in ten days and the curve resembles that for the toluene-evaporated and re-moistened soil, although the action is slower. But in soil left air-dried disappearance is much slower (Table XIII, Fig. 5) than in re-moistened soil, 30 per cent. of the phenol still remaining after 35 days; the curve is different in character, being slightly concave downwards, indicating conditions progressively inhibitory to the agent effecting the disappearance.

#### DISAPPEARANCE OF THE CRESOLS IN THE SOIL.

The cresols disappear in the soil as rapidly as phenol. In Tables XIV, XV and Fig. 6 are given data for the disappearance of *m*-cresol in Barnfield "dunged" and unmanured soils. The curve for the unmanured soil is practically a straight line as in the disappearance of phenol in the same soil: the same agency probably operated in both cases.

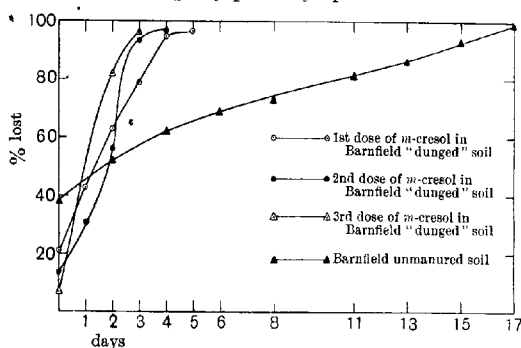


Fig. 6. Showing rate of disappearance of *m*-cresol in soils.

Table XIV.

*Disappearance of three successive doses of m-cresol in Barnfield dunged soil.*

Wt of soil = 100 gms. Wt of *m*-cresol = 54 mgms.

Period of contact with soil	% of <i>m</i> -cresol lost (successive doses)		
	1st dose	2nd dose	3rd dose
20 minutes	21.11	13.85	7.37
1 day	42.65	31.00	—
2 days	63.44	56.37	82.15
3 "	79.22	93.93	96.37
4 "	94.19	96.35	—
5 "	96.28	—	—

Table XV.

*Disappearance of m-cresol in Barnfield unmanured soil.*

Wt of soil = 100 gms. Wt of phenol = 54 mgms.		
Period of contact with soil	mgms. m-cresol recovered	% m-cresol lost
20 minutes	33.3	38.33
2 days	25.69	52.43
4 "	20.49	62.06
6 "	16.76	68.88
8 "	14.64	72.88
11 "	9.93	81.61
13 "	7.2	86.66
15 "	3.65	93.24
17 "	0.45	99.17

## PART II.

## INSTANTANEOUS DEPHENOLISATION IN SOIL.

Reference has been made to the remarkable fact that added phenol could not be completely recovered, even by starting the distillation within 15–20 minutes of the addition of phenol. Drastic measures like the use of superheated steam and the addition of acids were alike ineffective.

The initial loss takes place in all soils, fresh or air-dry, but not in pure sand, from which complete recovery of the phenol is obtained. Autoclaving the soil at 130° C. for half an hour or steaming it at 100° C. for one hour are the only two treatments yet found to destroy the cause of the initial loss.

Several possible explanations have been investigated. The possibility that phenol enters into simple chemical combination with some of the soil bases seems ruled out by the fact that acids do not increase the recovery. Biological decomposition seems equally insufficient to account for the facts. The rate of the subsequent disappearance is far slower than that of this reaction in which 20–30 per cent. of the added phenol is lost in 20 minutes. Moreover, Barnfield unmanured surface and sub-soil in which disappearance of phenol is slowest show the highest initial loss; while air-drying which exerts a profound influence on the subsequent biological decomposition does not interfere with the initial loss.

There is also the possibility of the absorption of phenol by other organisms to which it acts as a poison. Such an action is not improbable, but it cannot be the sole cause because the loss is greater in Barnfield

unmanured, poor in soil organisms, than in Barnfield "dunged" soil, in West Barnfield soil and in rich cucumber-house soil—all with a rich flora and fauna; and it is greater in the subsoil of Barnfield unmanured plot than in the surface (Table XVI). These facts suggest that the cause of the loss is probably not biological at all.

Adsorption of phenol by soil colloids seems also insufficient explanation since this should be reversible. A general relationship, however, seems to exist between the clay content of the soil and its power of instantaneous dephenolisation.

The effect of acids on the reaction is marked and very suggestive. Addition of dilute sulphuric acid rather augmented the loss: stronger sulphuric acid (15 c.c. of 50 per cent. by volume per 100 gms. soil) raised the loss to 90 per cent. Phenol sulphonic acid was completely decomposable by steam under the conditions of the experiment. Obviously, therefore, the tremendous loss of phenol in soil treated with sulphuric acid is due to some change—chemical or physical, or both—induced in the soil by the action of sulphuric acid and not to any formation of phenol sulphonic acid. This action takes place in autoclaved soil also. The amount lost when sulphuric acid is added to the phenolised soil is much greater than that lost when phenol is added to the acidified soil. The writer tentatively suggests that the cause of the loss due to the treatment of the soil with sulphuric acid differs fundamentally from that of the initial loss occurring in untreated soils and that sulphuric acid, autoclaving and steaming, are alike able to destroy the cause of the other type of loss.

*m*-Cresol appears to behave like phenol with or without sulphuric acid.

Table XVI.

*Loss of Phenol in air-dry soils.*

100 gms. air-dry soil }  
50 mgms. phenol in 2 c.c. } in each case.  
Distillation starting 20 minutes after phenolisation.

Soils	Mgms. phenol recovered	% phenol lost	Remarks
West Barnfield ...	42.08	15.84	Lightest soil at Rothamsted
Barnfield unmanured (top 3 inches)	39.12	21.76	Heavy loam—heaviness no doubt increasing with depth.
Barnfield unmanured (top 6 inches)	36.78	26.44	
Barnfield unmanured (9–15 inches)	35.50	29.00	

*The instantaneous loss of phenol in air-dry soils varies between 15 and 30 per cent. of the added phenol, according to the heaviness of the soil. Results in support of this statement are given in Table XVI.*

The amount of loss does not appear to be determined by the period of contact with soil prior to the commencement of distillation. Usually the period was about 20 minutes. Table XVII, however, shows the results when Barnfield unmanured soil (top 3 inches air-dry) stood in contact with phenol for 1-45 minutes before the commencement of distillation. When allowance is made for the lack of uniformity in the composition of the soil, the period of contact will seem to exert little influence on the magnitude of loss.

Table XVII.

*Effect of period of contact on the loss of Phenol.*

100 gms. Barnfield unmanured air-dry soil (top 3 inches).  
50 mgms. phenol.

Period of contact with soil	Mgms. phenol recovered	% phenol lost
1 minute	41.82	16.36
5 minutes	40.57	18.86
10 „	37.12	25.76
20 „	39.12	21.76
45 „	38.57	22.86

Table XVIII.

*Loss of Phenol in varying concentrations, the absolute weight being constant.*

100 gms. Barnfield unmanured soil air-dry (top 3 inches).  
50 mgms. phenol.

Distillation starting 20 minutes after phenolisation.

		Mgms. phenol recovered	% phenol lost
50 mgms. phenol in 2 c.c.		38.58 39.66	39.12 21.76
„ „ 4 c.c.		38.52 38.34	38.45 23.10
„ „ 6 c.c.		38.54 38.98	38.76 22.48
„ „ 8 c.c.		40.00 38.48	39.24 21.52
„ „ 10 c.c.		42.02 39.54	40.78 18.44
„ „ 20 c.c.		40.99 40.23	40.61 18.78

The amount of initial loss of phenol seems to depend on the total quantity presented to the soil, and not on its *initial* concentration.

To successive 100 gms. lots of Barnfield unmanured air-dry soil (top 3 inches) was added 50 mgms. phenol in 2, 4, 6, 8, 10 and 20 c.c. of the solution. The losses in the various cases are of similar magnitude. A later experiment (p. 156) indicates that loss is not complete until the steam has saturated the soil to a definite degree of moistness, in which case the ultimate concentration of phenol is the same in the various cases.

When, however, the absolute amount of phenol varied the loss also varied with it, and this held true of *m*-cresol also.

To lots of 100 gms. of air-dry soil of West Barnfield was added respectively 12.5, 25, 50 and 100 gms. of phenol: in another experiment 100 gms. lots of Barnfield unmanured soil (top 6 inches) were treated with quantities of phenol ranging between 3.125 and 100 mgms., the phenol being applied in 2 c.c. of the solution in each case. The distillation in each case was started 20 minutes after phenolisation (Table XIX).

Table XIX.

*Effect of concentration on the extent of loss of Phenol.*

100 gms. air-dry soil. 2 c.c. phenol solution.				
Mgms. phenol used	West Barnfield soil		Barnfield unmanured	
	Mgms. phenol recovered	Mgms. phenol lost	Mgms. phenol recovered	Mgms. phenol lost
3.125	—	—	2.11	1.015
6.25	—	—	4.37	1.88
12.5	10.37	2.13	8.39	4.11
25	20.94	4.06	17.31	7.69
50	42.08	7.92	36.78	13.22
100	91.46	8.54	83.9	16.1

Table XX.

*Effect of concentration on the extent of loss of m-cresol.*

100 gms. Barnfield unmanured air-dry soil (top 3 inches).  
4 c.c. *m*-cresol solution.

Mgms. <i>m</i> -cresol used	Mgms. <i>m</i> -cresol recovered	Mgms. <i>m</i> -cresol lost
6.25	2.69 1.9	2.29
12.5	6.68 6.46	5.93
25	16.69 14.87	9.22
50	32.18 31.29	18.27



Up to the point where 50 mgms. are added in 2 c.c. the extent of the loss of phenol appears to be roughly proportional to the concentration of the phenol solution.

Similar results have been obtained with *m*-cresol (Table XX).

The magnitude of the loss of phenol and *m*-cresol in varying amounts of soil varies directly, sometimes almost proportionately, with the weight of soil used. The duplicates agree closely except where 200 gms. of soil were used—a fact for which we are unable to account.

The results suggest that the whole of the soil used comes into play in determining the loss of phenol, which implies that the loss is not complete until the phenol has been distributed over the whole mass of the soil as a result of the uniform moistening of the soil by the intruding steam at the initial stage of distillation.

Experiments with *m*-cresol led to similar results.

Table XXI.

*Loss of Phenol and m-cresol in varying amounts of soil.*

Barnfield unmanured air-dry soil (top 3 inches).					
50 mgms. phenol or <i>m</i> -cresol in 2 c.c. in each case.					
Gms. soil	Phenol		<i>m</i> -Cresol		
	Mgms. phenol recovered	% phenol lost	Mgms. <i>m</i> -cresol recovered	% <i>m</i> -cresol lost	
12.5	47.81 } 47.62	4.76	47.12 } 47.17	5.66	
	47.42 }		47.22 }		
25	45.57 } 45.66	8.68	46.21 } 44.79	10.42	
	45.75 }		43.37 }		
50	42.99 } 43.23	13.51	38.34 } 39.4	21.2	
	43.47 }		40.47 }		
100	38.58 } 39.12	21.76	32.18 } 31.73	36.54	
	39.66 }		31.29 }		
	36.73 }				
200	25.27 } 29.28	40.44	25.22 } 22.96	54.08	
	30.14 }		20.71 }		
	26.96 }				
300	25.00 } 25.21	49.58	—	—	
	25.42 }				

The effect of autoclaving and steaming on the soil is to destroy the agent causing loss of phenol and of *m*-cresol, and practically 100 per cent. recovery is effected from soils thus treated.

Except in a few cases no loss of phenol has been observed in soil previously ignited in a muffle.

## SUMMARY.

1. Two colorimetric methods of estimation of phenol and the cresols in soil have been developed,—one for small quantities down to 0.01 mgm. in 50 c.c. of the soil distillate, and the other for larger amounts of the substances.

2. It is found that, even by starting the distillation of the soil within 20 minutes of the application of phenol, a 100 per cent. recovery of the added phenol cannot be effected, except in the case of autoclaved or steamed soil. This instantaneous disappearance is followed by a further loss, slower and different in character.

Phenol and the three cresols disappear, with more or less ease, in all soils examined.

3. The slow disappearance is apparently largely brought about by micro-organisms which are capable of decomposing phenol. There appears, however, to be some non-biological action also, since the disappearance in unmanured soil poor in micro-organisms, besides being much slower than in manured soil, is altogether different in character.

4. Autoclaving the soil at 130° C. for 20 minutes destroys the cause or causes of disappearance altogether, but the action proceeds, although much more slowly than in untreated soil, in the presence of a considerable amount of toluene and mercuric chloride. Further support is thus given to the view that a non-biological action accompanies the biological decomposition.

5. Partial sterilisation by treatment with toluene which was evaporated before the addition of phenol increases the rate of loss, but steaming does not.

6. The disappearance takes place even in soil air-dried to 2.4 per cent. moisture, but it is extremely slow compared with the rate in normal soil.

7. By applying a number of successive doses of phenol to the same soil it has been found that each dose disappears at a higher rate than the preceding one. This is entirely in accordance with an action mainly biological in character. The same effect has been observed in the case of *m*-cresol.

8. Two types of bacteria have been isolated from soil which are able to decompose phenol in culture solutions containing mineral salts and phenol. A third type capable of decomposing *m*-cresol has also been isolated from soil. A detailed study of the isolated organisms is being made in the bacteriological laboratory.

9. The instantaneous disappearance of phenol occurred in all soils examined. Its cause appears to be non-biological, but its exact nature has not yet been elucidated. There is reason to believe that the loss varies with the clay content of the soil. The loss does not appear to be materially affected by the period of contact of phenol with the soil prior to distillation, and is presumably complete only when the soil (air-dry) has been uniformly moistened with steam. Up to a certain point the loss is proportional to the concentration of the phenol solution.

10. The factor causing the loss is destroyed by autoclaving the soil at 130° C. for half an hour or by steaming it at 100° C. for one hour.

The treatment of the soil with sulphuric acid (50 per cent. by volume) either before or after addition of phenol greatly augments the loss, which may amount to 90 per cent. in case of phenol and 96 per cent. in case of *m*-cresol. This loss is not affected by autoclaving the soil.

11. Thus there appear to be at least three processes whereby phenol disappears from soil: one biological, one instantaneous chemical or physical and a third slow, chemical or physical. The mechanism of these changes, however, has not been elucidated.

This work was carried out during my tenure of the Earl Grey Memorial Fellowship awarded me by the Council of Armstrong College to whom and also to the Lawes Agricultural Trust I wish to express my thanks for facilities for carrying out this work.

My best thanks are due to Dr E. J. Russell at whose suggestion the investigation was undertaken and under whose direction the work has been carried out: and to Mr H. G. Thornton for assistance on the bacteriological side.

*(Received December 20th, 1920.)*

# INHERITANCE IN BARLEY.

## II. THE AWN AND THE LATERAL FLORET.

[For I. see Bibliography (1).]

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SOME interesting contributions to this subject have appeared during the past three years. They contain somewhat discordant ideas and it has therefore been concluded that the present time is suitable for a brief review of the whole matter and for the publication of further experimental results. A striking susceptibility to environmental change is evinced by both the awn and the lateral floret, and it is part of the purpose of this paper to describe the nature of the fluctuations involved and to indicate the extent to which they militate against the reliable determination of genetic ratios.

A retrospect of genetic investigation—over the period 1900 to 1920—compels the question: "What has been discovered and where does the knowledge lead?" The bulk of the investigation has, quite naturally, taken the form of the establishment and confirmation of "genetic ratios." Segregation, as a bare phenomenon, has been proved to characterise the processes of reproduction but little has been achieved in the

elucidation of the precise morphological and physiological "characters" which behave as "units" when segregation is in process. The pre-Mendelian systematist was content to define floret-characters almost exclusively in two general terms—present (i.e. apparent to the eye) and absent. So long as the duty of building up a satisfactory general confirmation of Mendel's original discovery yet remained, genetics had no need to attempt greater precision than this. That such a need exists now and that the quest of ratios based upon simple "eye-judgment" characters may be in some cases disappointing and unreliable, is one of the indications of the experimental work to be described. Some attention has, in consequence, been devoted to that aspect of the observations which suggests—for these two experimental characters at any rate—the desirability of trying to raise one of the common present-day forms of genetic experiment to a different plane, a plane upon which genetics and plant physiology co-operate very closely.

To appreciate the different aspects of the situation it is necessary to devote a certain amount of consideration to the purely botanical nature of the experimental characters and for this reason, in the case of each of them, a descriptive paragraph has been set apart.

#### § I. THE AWN IN BARLEY CONSIDERED AS A GENETIC CHARACTER.

In many genera of the Gramineae the outer palea (also called the flowering glume or lemma) and, more rarely, the glume (also called the empty glume) bears a bristle-like appendage. This is called the awn. The cultivated forms of the genus *Hordeum* (= the barleys) bear an awn on the *glume* which is insignificant but—save in certain cases described below—the awn of the outer palea is very long and prominent. It gives to the ear the appearance of having a long "beard." Reference to the awn in what follows is always to the awn of the outer palea of the floret.

Barleys which are commonly accepted as "natural" varieties fall into groups whose awn characters are as follows:—

*H. hexastichum* (6-row and 4-row barleys) with long awns on both median and lateral florets [both sets of florets form fertile grains].

*H. intermedium* (*H. i. Haxtoni* and *H. i. transiens*) with long awns on the median florets, but with lateral florets which, though generally setting grains, have blunt tips to their outer paleae.

*H. distichum* is like *H. intermedium* in awns. The lateral florets never set grains and have smaller paleae than in *H. intermedium*.

*H. decipiens* is like *H. distichum* save that the paleae of the lateral florets are much reduced, being, in fact, mere vestiges.

There is a group of barleys indigenous to parts of the Himalayas, which differs from the other forms of the genus *Hordeum* in that it has "hoods" or "caps" (the Kaputzer of Körnicke) in the place of awns. In a "hood" may occur a small flower. Two downward sloping lateral projections from the base of the hood lend a triangular appearance and hooded barleys are commonly known as "trifurcate" forms. Among the naturally occurring cultivated barleys, *H. hexastichum* alone shows "trifurcate" varieties, but trifurcate "distichums," etc., can readily be bred.

That the awn and the hood are simply different developments of the same organ is shown by their broad similarity and by the inheritance results recorded by Biffen(7). Certain of his  $F_1$  and  $F_2$  plants in Awned  $\times$  Trifurcate crosses had a hood borne on a short awn. To some extent, therefore, the awn and the hood are teratological, there being a series of possible developments—awn, hood, and awn-cum-hood. In such a character sharp fluctuations are perhaps to be expected. They occur in a most baffling manner and a brief account of them must be given later.

In the accepted "natural" races of barley wide fluctuations in awn-length do not appear. When, however, hooded and awned forms are crossed, sorting into types may be impossible owing to the vagaries of the hood-cum-awn form [cf. Biffen(7)]. To further the investigation of awn-inheritance "awnless" forms of barley must be used—forms in which the outer paleae of median and lateral florets alike bear neither awn nor hood. Two such forms are known to exist and they merit close attention. The first is a variety called "Nogenasi" described by Ikeno(8). It is a 6-row form. Apparently it is a "natural variety" in Japan. How it has arisen is not clear and, as will later be shown, its existence is of some botanical interest [cf. § III, p. 165, below]. Although it must be classed as unique—beardless 6-row—it by no means represents a "clean-cut" character, a perfect "absence." Ikeno's own words best illustrate the nature of the awn fluctuation [*loc. cit.* p. 263]: "Nogenasi où les arêtes font en général défaut; on y voit cependant très fréquemment la production des arêtes courtes chez un certain nombre des épillets appartenant au rang moyen et il importe de remarquer ici que même dans tels cas les arêtes ne se produisent jamais chez ceux placés à la partie supérieure de l'épi et qu'elles n'ont jamais une longueur suffisamment grande pour que leur extrémité supérieure puisse atteindre le niveau des épillets placés au sommet de l'épi." In connection with the final clause of this quotation it is to be noted that the protrusion of the awns beyond the tip of the ear is no guide (in an  $F_2$ ) to awn-length type unless there

is absence of segregation of rachis-segment length. A set of awns which makes a "dense" ear look half-bearded, presents quite an unimpressive appearance on a very long, lax ear. Segregation of rachis length is commonly encountered in barley crosses. It has greatly added to the complexity of the awn-inheritance problem.

The other "awnless" barley is *Hordeum distichum inerme* (hereinafter "Inerme"). Rimpau(9) was the first to breed it—from the cross:

Steudel's Gerste $\times$ Gabelgerste	
(H. decipiens v. Steudelii) $\times$ (H. hexastichum v. trifurcatum)	
= median florets fully awned;	= all florets setting grain and
laterals vestigial	all bearing a hood

One of a number of segregates from this cross, "Inerme," was awnless median florets and semi-deciens laterals. Its grain is poorly filled and rich in nitrogenous compounds, its straw deep chrome in colour and of marked strength. These characters have led to its employment in the endeavour to raise a "feeding barley." Usually the outer paleae of the median florets are bluntly rounded but fluctuation is common. "Awns" of 2-4 cm. in length may occur especially on late tillers of a plant. Quite commonly on one plant may be found—in order of earliness—the absolutely awnless ear, the ear with very short scurs, and the ear with 2-4 cm. long awns. For some unknown reason "Inerme" types extracted from  $F_2$ 's of certain Inerme  $\times$  Awned crosses, fluctuate far more violently in respect of "awn" than does the pure parental Inerme itself. It seems that the "Inerme potentiality" responds readily to environmental differences (e.g. changes in time of emergence of the ear from the sheath) and that, placed in the novel circumstances afforded by various genetic types extracted from  $F_2$ 's, the "potentiality" is even more responsive than in those afforded by the pure strain known as "Inerme."

The photographs included in this paper illustrate the range of awn fluctuation, which has been met in a number of Inerme crosses. Specimen ears of a number of  $F_2$  plants were preserved for comparison with  $F_3$  because of the obvious magnitude of possible fluctuations.

Fig. I  $\left\{ \begin{array}{l} (a) \text{ An } F_2 \text{ plant with awns 2.5 cms. in length.} \\ (b), (c), (d) \text{ } F_3 \text{ plants from the progeny of (a). All are completely awnless as were} \\ \text{all the eighteen members of this } F_3 \text{ family.} \end{array} \right.$   
The cross was *H. i. Hartoni*  $\times$  *H. d. inerme*.

Fig. II. Ears from one  $F_3$  plant of the cross *H. h. parallelum*  $\times$  *H. d. inerme*.

Fig. III. Ears from one  $F_3$  plant of the cross *H. d. (v. Chevallier)*  $\times$  *H. d. inerme*.

Figs. II and III illustrate tiller to tiller fluctuations on a single plant. Körnicke(16) has recorded a great number of interesting cases of awn fluctuation, the most striking being Hoffmann's case [see (16), p. 135, vol. 1], in which a strain of 2-row barley bore in some years awned ears and in others awnless ones.

The "awn" of barley is thus a most difficult character to observe in genetic work. Classification of an  $F_2$  is usually impossible save by the raising of the  $F_3$  families and even if this be done the way to reliable sorting is far from open. Judgment has to be based on the  $F_3$  family as a whole and due regard has to be paid to the fact that late tillers of genetic "Inermes" may bear short awns. If the  $F_3$  family be large, classification can be effected with confidence, but families of only one, two, or three plants occur. It is dangerous to throw them away and, perhaps, equally dangerous to sort them.

## § II. VIEWS UPON THE INHERITANCE OF THE AWN IN BARLEY.

Ikeno's observations(8) may be thus represented:

Kinukawa	×	Nogenasi
(Awned 6-row)		(Awnless 6-row)
<i>AA.EE.ii</i>		<i>aa.ee.II</i>

*A* and *E* have awn-producing powers, *I* inhibits awns. The  $F_2$  contained 321 plants and a complete  $F_3$  was grown from it. Of the  $F_2$  plants 4 gave entirely Kinukawa progeny and 6 entirely Nogenasi. The mean of 4 and 6 is 5;  $5/321 = 1/64$ ; therefore three factors are involved (as represented above). The  $F_2$  was further classified by judgment of its  $F_3$  families, but it was not found possible to attempt a sorting more elaborate than that represented by the following grouped types:

$$ii : Ii : II$$

This sorting gave the result:

$$69 : 164 : 88$$

The expectation on the three-factor theory is:

$$80.25 : 160.50 : 80.25$$

The error of 69/321 instead of 80.25/321 is about 2.2 times the probable error due to fluctuations of sampling.

What, in effect, has been accomplished is therefore a classification of the  $F_2$  plants (by means of a complete  $F_3$ ) on a basis which agrees—and not in a conspicuously accurate degree—with a theory of unifactorial difference between the awned and awnless parent forms.

So far the justification for the 3-factor theory rests solely upon the rather slender evidence that the number of true-breeding parental-type plants in  $F_2$  is 5/321 ( $4 + 6/2 = 5$ ). Further justification was sought by determining the proportions of  $F_2$  plants in the groups

$$ii : Ii : II$$



which bred true to type in  $F_3$ . The results were (in numbers of plants):

$$21 : 0 : 23$$

and the expectation:

$$17.25 : 0 : 22$$

Here the agreement is certainly good. The 3-factor theory emerges better from this sub-proof than from the proof-general already considered.

Since the behaviour of partially and completely awnless forms is so erratic, more ample information is desirable for nothing is said as to the numbers of plants in the  $F_3$  families, nothing is said as to the existence of tiller to tiller fluctuations and but little is said as to plant to plant fluctuations in a genetically uniform population. Dominating all these considerations is yet another—personal bias. In respect of this, investigators doubtless differ; but in the handling of a considerable number of  $F_2$ 's and  $F_3$ 's involving different awn types it has never been possible to remove the fear of "bias." No method of check—e.g. by co-variant characters, such as might be based on an exact knowledge of the homology of the awn—has so far been devised to vindicate awn-sorting by eye. Safety rests only in very large numbers. That 4 plants (no more and no less) out of 321 breed true to the awn-characters of one of the parents is, in view of the circumstances which have been alluded to, a most difficult decision to make even if all four gave large  $F_3$  families.

v. Ubisch<sup>(10)</sup> has worked very extensively upon the inheritance of barley awns. Comment has been made in a previous paper (Engledow (1), p. 102) upon the recognition of palea types such as "Spitz," and "Spitz verlängert." Paragraphs III and IV of his (v. Ubisch) paper deal respectively with the inheritance of beard-length and the "korrelation" (in the sense of coupling or linkage) between density of ear and beard-length.

In his own words, beard-length is thus defined: "Als Grannenlänge ist hier die Differenz Ähre mit Granne—Spindellänge bezeichnet." That is to say the length of the beard is judged by the greatest amount of protrusion of any one of its constituent awns beyond the tip of the rachis (Spindel) of the ear. How profoundly the density of the rachis is responsible for this amount of protrusion has been explained already. The data of v. Ubisch consist entirely of "protrusion" measurements. Not to observe the three variables obviously concerned—lengths of individual awns, rachis length, and number of internodes of the rachis—seems an indirect treatment of the problem. A "korrelation" (coupling) is established between density of rachis and beard-length, but it is perhaps a justifiable comment that since beard-length depends to some extent

upon rachis-density something in the nature of a correlation is almost bound to be observed between these two variables. In the absence of data of parental,  $F_1$ ,  $F_2$  and  $F_3$  rachis characters, further examination of the results is impossible.

### § III. SOME EXPERIMENTAL RESULTS ON THE INHERITANCE OF THE AWN IN BARLEY.

The results to be described are all derived from a series of crosses of various "awned" barleys with "Inerme." Although raised primarily for plant-breeding purposes, the  $F_1$ 's,  $F_2$ 's and  $F_3$ 's have been utilised as far as possible for genetic observation. In many cases it has not been possible to raise a complete  $F_3$ , for every such  $F_3$  (containing 5 8000 plants) involves a great deal of work and there were 27 complete  $F_2$ 's to be considered. The  $F_3$  growings—partial and complete—were arranged so as best to meet, within the limitations of available time, the requirements of both plant-breeding and genetic experiment. It is clearly explained in every cross dealt with below, whether the evidence is that of a partial or a complete  $F_3$ . The  $F_2$  results in all cases are derived from complete  $F_2$ 's. In harvesting all the  $F_2$ 's every tiller of every plant was gathered, a separate bunch of ears was thus made for every plant. Thus all possible precautions were introduced to counteract the danger of the misleading "tiller to tiller" fluctuations.

The crosses fall into groups characterised by the botanical classes of the awned parents and the results are recorded under these groups.

#### GROUP I. *H. HEXASTICHUM* (AWNED) × INERME.

Cross *E. 30* = *H. hexastichum* (var. *Praecox*) × *Inerme*.

*Praecox* is a lax-eared 6-row and in other characters connected with the awn and lateral floret it appears to be typical of the *H. Hexastichum* class. *Inerme* has been fully described in § I (above).

**F**<sub>1</sub> = 14 plants. The awns of the median florets were of about "half-length" so that none of their tips protruded much above the level of the tip of the rachis of the ear. The lateral florets were occasionally fertile (i.e. they set a grain), there being one or two such fertiles on every ear. All the  $F_1$  plants appeared to answer the above description and no more precise account of awn characters was possible. The grain from eight of the fourteen plants was sown—the remaining plants being kept for reference—and in the tables below these plants are designated *E. 30.A*, *E. 30.B*, ... *E. 30.H*.

**F**<sub>2</sub> = 688 plants. Repeated independent sortings were made, every plant, however small and poor, being included. The results of the repeated sortings did not agree. Quite clearly, if a number of awn-types existed, the eye was unable to detect them with certainty. Only one classification led to consistent results on repetition—that of ( $\alpha$ ) plants in all respects resembling the parent Praecox and ( $\beta$ ) plants not resembling it. The latter class contained many degrees of awn and lateral floret form. If the classes be designated 6-row and non-6-row the results may be thus expressed:

Table I. *F*<sub>2</sub>.E.30.

	6-row		Non-6-row		Total
	Number	%	Number	%	
Observed ... ..	164	23.84	524	76.16	688
Expected (1:3 basis)...	172	25.0	516	75.0	688
Actual errors ... ..	—	1.16	—	1.16	—
Standard error of expectation <sup>1</sup>	—	1.65	—	1.65	—

Full-length awns appeared to be present only in plants of 6-row type; and plants which set six rows of grain appeared always to have full-length awns. It is, therefore, concluded from the *F*<sub>2</sub> evidence that the two characters, (i) six rows of grains set on the ear, (ii) full-length awns, are controlled together and that the control is unifactorial (the above 3:1 ratio is statistically satisfactory). No further deduction could be made from the *F*<sub>2</sub> since the two-fold classification was alone possible.

Of the 688 *F*<sub>2</sub> plants, 522 provided a reasonable amount of grain likely to be germinable. Owing to loss by wire-worm only 505 *F*<sub>3</sub> families were finally raised and harvested.

**F**<sub>3</sub> = 505 families. These families were sorted into three classes, viz.:

( $\alpha$ ) All plants in the family fully awned. The plants proved to be all of parental Praecox (6-row) type.

( $\beta$ ) Families showing segregation for the awn. 6-row type, Inerme type, and partially bearded types with laterals of varying forms were found in these families.

( $\gamma$ ) All plants as "awnless" as the Inerme parental form.

<sup>1</sup> The standard error is thus calculated. Let  $p$  and  $q$  be the expected percentages (here = 25 per cent. and 75 per cent.) and  $n$  = number of observations. Then standard error of expectation as a percentage =  $\sqrt{\frac{p \cdot q}{n}}$ . The probable error is, of course, obtained by multiplying the standard error by 0.6745.

The results of the sorting are contained in Tables II and III.

Table II.  $F_3.E.30$ .

$F_1$ progenitors of $F_3$ families	Classification of $F_3$ families			Total
	( $\alpha$ )	( $\beta$ )	( $\gamma$ )	
$E.30.A$	18	47	17	82
$E.30.B$	10	34	9	53
$E.30.C$	23	42	19	84
$E.30.D$	6	17	13	36
$E.30.E$	15	36	21	72
$E.30.F$	22	42	24	88
$E.30.G$	11	12	7	30
$E.30.H$	15	29	16	60
Total	120	259	126	505

Table III. *Résumé of Table II with errors, etc.*

	Pure 6-row families i.e. ( $\alpha$ )		Splitting families i.e. ( $\beta$ )		Pure Inerme families i.e. ( $\gamma$ )		Total
	Number	%	Number	%	Number	%	
Observed ...	120	23.76	259	51.29	126	24.96	505
Expected (1:2:1 basis) ...	126.25	25.00	252.50	50.00	126.25	25.00	505
Actual errors ...	—	1.24	—	1.29	—	0.04	—
Standard errors of expectation* ...	—	1.93	—	2.22	—	1.93	—

\* See footnote to p. 166 (above).

Since the classification of 505  $F_3$  families is equivalent to that of an equal number of  $F_2$  plants, it is concluded that the  $F_3$  results support those of the  $F_2$ . There is a one-factor difference for "awn" between Praecox and Inerme.

Discussion of the significance of the apparent inseparability of (i) 6 rows of grains set on the ear, and (ii) full awns, is postponed to § VI below, where data are given concerning the lateral floret types of this cross. Meantime the requirements of statistical safeguards have been provided (see Table III) and the one-factor awn may be accepted.

Cross  $E.28 = H. hexastichum$  (v. parallelum)  $\times$  Inerme.

Parallelum is a dense-eared 6-row barley.

**F**<sub>1</sub> = 5 plants. Like those of  $F_1.E.30$  described above, the ears were "half-awned" in the median florets and awnless in the laterals.

**F**<sub>2</sub> = 235 plants. As in  $F_2.E.30$  (above) a two-fold classification was all that could be performed consistently. Results were obtained as set forth in Table IV.

Table IV.  $F_2$ . E.28.

	6-row		Non-6-row		Total
	Number	%	Number	%	
Observed ... ..	55	23.40	180	76.60	235
Expected (1 : 3 basis) ... ..	58.75	25.00	176.25	75.00	235
Actual errors ... ..	—	1.60	—	1.60	—
Standard error of expectation*	—	2.82	—	2.82	—

\* See footnote to p. 166 (above).

Thus, again, a unifactorial awn-difference between a 6-row barley and Inerme is indicated. Again, too, the 6-row habit and the full-awn habit prove to be inseparable.

$F_3$  = partial only (for strong-strawed types).

#### GROUP II. *H. intermedium* × INERME.

Cross E.37 — *H. intermedium* (var. Haxtoni) × Inerme.

Haxtoni is a typical "intermedium" barley (see § I above) with a lax ear [i.e. median florets fully awned, laterals usually setting grain, but with blunt ended outer paleae].

$F_1$  = 10 plants. The awns of the median florets were of "half-length," being never more than 2-2.5 ins. in length. The lateral florets were, without exception, quite awnless.

$F_2$  was an unsortable complex. Many of the plants were imperfectly developed and very considerable tiller-to-tiller (same plant) fluctuations were found. In a few cases, very late tillers had lateral florets whose outer paleae bore short awns. Of the total  $F_2$  579 plants provided seed.

$F_3$  = 558 families (from 579 separate sowings). Disregarding the lateral florets—they are fully described in § VI below—the families were sorted into three classes, viz.:

(α) All plants in the family fully awned.

(β) The plants of the family showing "splitting" for awns (three types of awn were seen, viz.: full awns as in Haxtoni, half-awns [various, but not separable degrees of awning], and awnless like Inerme).

(γ) All plants in the family as awnless as Inerme.

"Full-awn," "half-awn," and "awnless" types are shown in Figs. VII, VIII and IX respectively.

Tables V and VI give the results of the sorting:

Table V.  $F_3$ .  $E.37$ .

$F_1$ progenitors of $F_3$ families	Classification of $F_3$ families			Total
	( $\alpha$ )	( $\beta$ )	( $\gamma$ )	
$E.37.A$	19	36	25	80
$E.37.B$	18	53	29	100
$E.37.C$	35	78	34	147
$E.37.D$	15	33	14	62
$E.37.E$	11	20	6	37
$E.37.F$	10	27	7	44
$E.37.G$	25	36	27	88
Total	133	283	142	558

Table VI. *Résumé of Table V with errors, etc.*

	Families with full awns ( $\alpha$ )		Families splitting for awn ( $\beta$ )		Families of Inerme type ( $\gamma$ )		Total
	Number	%	Number	%	Number	%	
Observed ...	133	23.83	283	50.72	142	25.45	558
Expected (1:2:1 basis) ...	139.5	25.00	279.00	50.00	139.5	25.00	558
Actual errors ...	—	1.17	—	0.72	—	0.45	—
Standard errors of expectation* ...	—	1.83	—	2.11	—	1.83	—

\* See footnote to p. 166 (above).

Again, with statistical security, it may be said that the parent types—Haxtoni and Inerme—differ in respect of awn by one factor. By growing a complete  $F_3$  it has been possible to establish the existence of segregation in  $F_2$  on a 1:2:1 basis.

Between this cross and those described under Group I (above) an interesting difference exists. "Full awns" are not here inseparable from six rows of grain on the ear (i.e. from six rows as exemplified by the *H. intermedium* type of barleys). Quite definitely there occur plants having six rows of grain ("intermedium" pattern), but entirely devoid of awns. Such ( $F_2$ ) plants breed true in  $F_3$ . Further details are reserved to § VI below.

#### GROUP III. *H. DISTICHUM* (AWNED) $\times$ INERME.

For this group it was not possible, owing to restrictions upon time, to raise any complete  $F_3$ 's. The evidence is consequently less full than in the case of Groups I and II above.

Cross  $E.31$  = Russian Chevallier No. 6  $\times$  Inerme.

The Chevallier parent is typical of the well-known variety of which the most noticeable features are the lax ear and the woolly rachilla.

In other respects the variety is morphologically strictly comparable with the other kinds of the sub-variety "nutans" of *H. distichum*.

**F**<sub>1</sub> = 20 plants. The ears were even laxer than those of the Chevallier parent. Awns were confined to median spikelets in the "lower quartile" part of the ear and they were only  $\frac{1}{2}$  in.-1 $\frac{1}{2}$  ins. in length. All lateral florets were completely awnless.

**F**<sub>2</sub> = 631 plants and, for some unaccountable reason, sorting into awn types was comparatively a simple matter—in fact repetition gave consistent results. "Half-awned" forms seemed never to approach the full-awns and "Inermes" were definitely separable from the half-awns.

Table VII contains the results of the sorting, the classes being:

( $\alpha$ ) Fully awned like Chevallier.

( $\beta$ ) "Half-awned," i.e. not so fully awned as ( $\alpha$ )—an eye-judgment class, but in this cross, a practicably distinguishable one.

( $\gamma$ ) As awnless as Inerne.

Table VII. *F*<sub>2</sub>.E.31.

	Fully awned ( $\alpha$ )		Half-awned ( $\beta$ )		Inerne type ( $\gamma$ )		Total
	Number	%	Number	%	Number	%	
Observed ...	157	24.88	298	47.22	176	27.89	631
Expected (1:2:1 basis) ...	157.75	25.00	315.50	50.00	157.75	25.00	631
Actual errors ...	—	0.12	—	2.78	—	2.89	—
Standard errors of expectation* ...	—	1.72	—	1.99	—	1.72	—

\* See footnote on p. 166 (above).

The errors here are far more considerable than in the cases recorded above, but even so, it may be remarked that, when the general fit (all three classes,  $\alpha$ ,  $\beta$  and  $\gamma$ ) to expectation is tested by Pearson's  $\chi^2$  method (see (11) Table XII, p. 26) there proves to be a one in five chance of getting a worse fit by random sampling (i.e. by raising an *F*<sub>2</sub> of limited size).

**F**<sub>3</sub>. A large *F*<sub>3</sub> was grown for plant-breeding purposes. In an indirect way it afforded further evidence for awn-inheritance. Batches of ( $\alpha$ ), ( $\beta$ ) and ( $\gamma$ ) *F*<sub>2</sub> plants were sown. Before harvest those *F*<sub>3</sub> families were destroyed which proved not to belong to the group ( $\alpha$ ), ( $\beta$ ) or ( $\gamma$ ) in which they were growing—the *F*<sub>2</sub> progenitors had, of course, been incorrectly classified. The remaining families—those from *F*<sub>2</sub> plants

which had been correctly classified—were harvested. The facts proved to be:

(i) Of the  $F_2$  plants classified as fully awned, 119 were sown; but in six cases the  $F_3$  family proved not to be homozygous for "full-awn." Thus the proportion of accurately sorted plants in  $F_2$  was  $(119-6)/119 = 113/119$ . It may therefore be assumed that  $113/119 \times 157 = 149$   $F_2$  plants were certainly of the full-awn type.

(ii) Similarly  $\left(\frac{265-12}{265}\right) \times 298 = 285$   $F_2$  plants were certainly heterozygous for awn. [Their  $F_3$  families contained all the types ( $\alpha$ ), ( $\beta$ ) and ( $\gamma$ ).]

(iii) Similarly  $\left(\frac{133-27}{133}\right) \times 176 = 140$   $F_2$  plants were homozygous for the "awnless" character (i.e. of Inerme type).

Summarising, the results are as contained in Table VIII:

Table VIII.  $F_2.E.31$  as classified by the  $F_3$ .

		Fully awned ( $\alpha$ )		Half-awned ( $\beta$ )		Inerme type ( $\gamma$ )		Total
		Number	%	Number	%	Number	%	
Observed	...	149	25.96	285	49.65	140	24.39	574
Expectation	(1:2:1)	143.5	25.00	287.0	50.00	143.5	25.00	—
Actual errors	...	—	0.96	—	0.35	—	0.61	—
Standard errors of expectation*	...	—	1.80	—	2.08	—	1.80	—

\* See footnote on p. 166 (above).

The reliability of the results is limited by the method of working (explained above), but, within these limitations, a one-factor difference for awn between Chevallier and Inerme is proved with satisfactory numerical exactness.

Cross  $E.32 =$  Russian Chevallier No. 7  $\times$  Inerme.

A complete  $F_2$  was raised and sorted, results being as in Table IX.

Table IX.  $F_2.E.32$ .

	Fully bearded	Remainder	Total
Number	115	378	493
Percentage	23.3	76.7	—

No more detailed  $F_2$  classification was attempted and in  $F_3$  but twenty families were raised, these being the only selections possessing apparent economic value. As far as it goes, the evidence accords with that of Cross  $E.31$ —a unifactorial effect is again indicated.



Cross *E. 34* = Russian Goldthorpe No. 9 × Inerne.

This cross deserves to be placed on record for the reason that it furnished an instance of that neglected type of coincidence wherein are allied statistical security and error of inference. In the  $F_2$ , sorting appeared to be a safe undertaking, giving the result:

	Fully awned	Half-awned	Inerne
$F_2 =$	226	224	181

Here, clearly, two or three factors had to be invoked. Factors *A* and *B* were entrusted with the duty of producing awns and factor *I* with that of awn-inhibition. As is common in multifactorial explanations, assumptions were made, viz.:

- (i) The awn is completely inhibited if *II* be present.
- (ii) *Ii* does not noticeably inhibit the awn if *AA* be present.
- (iii) *AA*, *Aa*, *BB*, and *Bb* produce full-length awns in the presence of *ii*.

The factorial representation therefore became:

$$\begin{array}{lcl}
 F_0 & \text{Goldthorpe} \times \text{Inerne} & \\
 & (AA.bb.ii) \mid (aa.BB.II) & \\
 F_1 & \text{Half-awned} & \\
 & Aa Bb Ii & \\
 F_2 = ii (AABB + 2AABb + AAbb + 2AaBB + 4AaBb + 2Aabb + aaBB + 2aaBb) = 15 & & \\
 = 2IiAA (BB + 2Bb + bb) & & = 8 \\
 \quad \text{i.e. fully awned plants} = (15 + 8)/64 = 23/64 & & \\
 = 2Ii (2AaBB + 4AaBb + 2Aabb + aaBB + 2aaBb) & & = 22 \\
 \quad \text{i.e. half-awned plants} = 22/64 & & \\
 = II (AABB + 2AABb + AAbb + 2AaBB + 4AaBb + 2Aabb + aaBB + 2aaBb + aabb) = 16 & & \\
 = 2Ii.aa.bb & & = 2 \\
 = ii.aa.bb & & = 1 \\
 \quad \text{i.e. awnless plants} = 19/64 & & 
 \end{array}$$

The expectation is therefore:

	Fully awned	Half-awned	Awnless
$F_2 =$	23/64 = 35.94 %	22/64 = 34.37 %	19/64 = 29.69 %

Table X shows the observed results:

Table X.  $F_2.E. 34.$

	Fully awned ( $\alpha$ )		Half-awned ( $\beta$ )		Inerne type ( $\gamma$ )		Total
	Number	%	Number	%	Number	%	
Observed ...	226	35.83	224	35.49	181	28.68	631
Expected (theory above) ...	—	35.94	—	34.37	—	29.69	—
Actual error ...	—	0.12	—	1.12	—	1.01	—
Standard error of expectation ...	—	1.91	—	1.89	—	1.82	—

Statistically, therefore, the results are safeguarded.

Pressure of other work necessitated the growing of none but economically promising  $F_3$  families. Three groups of families were raised—from  $F_2$  plants sorted as fully awned, half-awned, and awnless respectively. That the  $F_3$  sorting had been unreliable became at once apparent. Families that should have been homozygously fully bearded were splitting for awn and so on. The reason for the error is believed to be the influence on "beard appearance" of the density of the rachis—an influence which was not fully realised until the  $F_3$  was handled. All  $F_3$  families which were clearly "splitting" for awn were put together and sorted plant by plant. Two types of beard were recognised:  $(\alpha)$ ,  $[(\beta) + (\gamma)]$  of Table X, and a sorting on this basis gave:

$$\begin{array}{ll} (\alpha) & : \quad [(\beta) + (\gamma)] \\ 68 & : \quad 195 \approx 1 : 3? \end{array}$$

The numbers are small but in the past even smaller ones have established a "ratio"; they serve, at any rate, to refute the *ABI* theory for which the  $F_2$  so accurately vouches.

The facts of this singular case are published solely as an illustration of the dangers which may attend the genetic investigation of what the human eye is sometimes satisfied to accept as a "plant-character."

Cross *E.41* = Plumage  $\times$  Inerme.

Plumage is a popular cultivated form of the broad-ear (dense rachis) type of *H. distichum*.

The  $F_3$  was treated in the manner described under Cross *E.34* above with the result:

$$\begin{array}{ll} (\alpha) & : \quad [(\beta + \gamma)] \\ 40 & : \quad 121 \end{array}$$

[The numbers are of single plants in  $F_3$  families which were clearly "splitting for awn."]

Here, again, is an indication of unifactorial difference for awn between an ordinary *H. distichum* barley and Inerme.

The derivation of Inerme in itself suggests this unifactorial difference. Rimpau's cross (described in § I above) may be represented:

$$\begin{array}{c} \text{Hooded 6-row} \times \text{Awned 2-row} \\ (AA\ bb) \quad | \quad (aa\ BB) \\ \hline F_2 \text{ contains } aa, bb = \text{Inerme} \end{array}$$

The existence of an awnless 6-row, "Nogenasi," (see Ikeno<sup>(8)</sup> and § I above) is of interest. For the "removal" of awns in 2-row barleys a

cross between "hooded" and "awned" forms proved to be necessary. Unless a hooded barley occurs in Japan and out-pollination of barleys is possible there, "Nogenasi" is, perhaps, to be regarded as a "mutation."

#### § IV. SOME GENERAL CONCLUSIONS UPON THE INHERITANCE OF THE AWN IN BARLEY.

The general conclusion from the experimental evidence which has been recorded is that the presence of a full-length awn is due to the homozygous presence in the plant of one factor: the intermediate or half-awn corresponds to the heterozygous presence of the factor; the absence of the awn to the absence of the factor. Between this conclusion and those of v. Ubisch and Ikeno there is the wide distinction of unifactorial as opposed to bi- and tri-factorial constitutions. That there exists a considerable range of apparent awn-forms may be regarded as *prima facie* evidence of multifactorial arrangement: but against this must be urged the certain existence of pure fluctuation. Figures I and III illustrate small extracts from a large and well-tried mass of similar material exemplifying fluctuation of the awn. In so far as the usual statistical safeguards are a guarantee of reliability the unifactorial explanation is acceptable, but Cross *E.34* (p. 172 above) makes it clear that fluctuation may, at times, conspire with statistics to mislead the investigator. It is to be noted that there is, save in Cross *E.31*, a constant small defect of the "fully awned" type. In amount the defect is not great enough to transgress the bounds of latitude allowed by "error due to random sampling," but it may nevertheless indicate an order of coupling which is so high that the single 1 : 2 : 1 ratio in  $F_2$  is but little disturbed.

Confirmation of some kind is needed in the case of the awn where—as with many other plant characters—fluctuation compels the investigator to regard his statistically acceptable "ratios" with apprehension. Repetition of the investigation is unlikely to be helpful and some other form of confirmation has to be sought. It is well known that in some plants and animals, there exist a number of features any one of which might be—some actually have been—looked upon as separate plant-characters, but all of which are transmitted *en bloc* in inheritance. "Characters" of this kind may conveniently be called "genetic inseparables." Doubtless they are simply separate manifestations of one real "plant-character," of a single biological process. Examples of "genetic inseparables" have been recorded *inter alia* in oats [Surface(12)], in oats [Zinn and Surface(13)], in wheat [Engledow(14)], and in barley [Engle-

dow<sup>(1)</sup>]. In *Drosophila* the same phenomenon is well exemplified, and Morgan and his collaborators [(15) pp. 209 *et seq.*] have already emphasised its importance. If in the case of the awn in barley some character could be found which stood in the relation of a genetic inseparable, the behaviour of that character could be observed in order to test classifications based upon awn-forms. Considerable effort has been expended in the search for a character inseparable from the awn, but so far nothing has been found. It may be that such a one does not exist; but it may equally be simply that the search has been misdirected. Physiological anatomy should direct a search of this kind, but as far as the cereals are concerned this is a very neglected subject, having, in fact, scarcely reached its infancy. An appeal to pure morphology may be made to determine to what organ or kind of organ the awn is homologous, for in this way the search for a genetic inseparable (and therefore a co-fluctuant) of the awn might be approximately orientated. The view of Hackel<sup>(2)</sup> upon the homology of the awn seems still to enjoy general acceptance. He opined that the awn corresponded to the lamella of the ordinary foliage leaf, the outer palea similarly being a modified leaf-sheath. It is well known that the awn has chlorophyll tissue, vascular bundles and stomata—features which are common not only to foliage leaves in general, but to herbaceous stems in general as well—and it has been demonstrated that the combined awns of a barley plant perform a large proportion of the total transpiration of the plant [see Ziehl and Mikosch<sup>(3)</sup>, Schmid<sup>(4)</sup>, and Perlitus<sup>(5)</sup>]. Against this somewhat general evidence for Hackel's leaf-homology is that derivable from the "trifurcate" barleys. In them the awn is in the form of a "hood" inside which may be a flower. Perhaps, therefore, there is more justification for regarding the awn, not as a modified leaf blade, but as part of a modified stem. The outer palea may similarly be regarded as a leaf-like stem [similar to those in the familiar cases of *Lemna* sp., *Ruscus aculeatus*, *Semele* (*Ruscus*) *androgynea*, etc.].

It thus appears that although wide fluctuation makes it indispensable either to discover some co-fluctuant of the awn or so to understand its botanical significance that the fluctuations may be interpreted, yet the means to both of these ends are at present lacking. The conclusion is therefore drawn that, although unifactorial inheritance is quite probable, there should be further investigation upon awn inheritance. Such investigation should deviate from the familiar path of eye-sorting of  $F_2$ 's and  $F_3$ 's and strike out upon the comparatively untrodden one of physiological anatomy.

§ V. THE LATERAL FLORET IN BARLEY CONSIDERED  
AS A GENETIC CHARACTER.

Throughout the genus *Hordeum*, one type of inflorescence prevails. The "ear" is formed on a central rachis of flattened cross section and alternating up the two broad sides of this are "notches" or "nodes." Grouped at every node are three spikelets, so that the ear has six rows of spikelets. Every spikelet consists of two small glumes (in a few exceptional cases these are fused into one), inner and outer paleae, a rachilla, and sexual organs. The spikelet invariably contains only one flower. In all barleys (save *H. sylvaticum*, noted below) the median florets—i.e. the two rows which are placed one along the middle of each of the broad sides of the rachis—are large; they set large, well-filled grains. The lateral florets—i.e. the remaining four rows which are obliquely placed at the sides of the median rows—possess a very wide range of forms in the different species and sub-species. In *H. hexastichum* (see § I above) the laterals set grains, but these never attain the size of the median grains and are readily detected by the slight "twist" which they show. Like the medians the lateral florets possess an awn (or hood) on the outer palea. The *H. intermedium* barleys are capable of setting grains in their lateral florets, but not of bearing an awn. Actually, the percentage of grains set by the lateral florets in *H. intermedium* varies with the growing conditions. All other cultivated barleys and all wild ones save *H. sylvaticum* have lateral florets incapable (in general) of setting grains. The various forms of *H. distichum* and *H. decipiens* exhibit a series of different sizes and developments of the lateral floret. Hybridisation adds to this series. In a previous paper [Bibliography(1), pp. 98–100] there is an account of the types commonly found and Körnicke (18) describes in great detail the usual structure and many of the observed abnormalities of lateral florets. One of the wild barleys, viz. *H. sylvaticum* is unique in possessing small medians and large, fertile, laterals.

It is generally assumed that the lateral florets of those barleys whose medians alone set grains, are deficient in female reproductive organs only (*H. distichum*) or in both male and female (*H. decipiens*). Lateral florets of the size found in *H. distichum* (or the slightly larger more pointed forms of certain heterozygotes) are commonly assumed, wherever found to be staminate only: the extremely small laterals which characterise *H. decipiens* forms are likewise assumed to be entirely sexless. Size is, in fact, supposed to indicate stage of development of reproductive organs. This is a questionable supposition and the exceedingly puzzling

problem of "inheritance of lateral florets" is not likely to be solved without an attempt to separate "size" from "sexual development" in making observations. It has become quite clear that the sexuality of lateral florets cannot be properly studied without a detailed cytological investigation of the ear in successive stages of development. In the investigations here recorded a certain measure of success has attended the endeavour to distinguish "fertility" and "size" as separate characters but only by cytological study will final results be obtained. In, for example, "Archer" barley (a common cultivated form of *H. distichum*) stamens are certainly present and surrounded by the bases of their filaments is a small "organ" which nothing but careful sectioning can prove or disprove to be an ovary. The assumption that Archer laterals are staminate only, rests entirely on the quite unreliable judgment of the naked eye.

In attempting to classify the plants of an  $F_2$  or the families of an  $F_3$  (an  $F_3$  family implying, of course, the progeny of a single  $F_2$  plant) consideration has to be given to both size and general evidence of potential fertility of the lateral florets. The circumstances vary with the individual crosses concerned and in the paragraph of experimental results which follows, an account is given in the case of every cross, of the basis of judgment which was adopted.

The lateral florets of "Inerme" must be described since that form was one of the parents in all the crosses concerned. These laterals are in some respects unique. Their most noticeable feature is the relatively great length of their glumes [see Fig. V], whose actual "tips" (excluding the short "glume-awns") attain the level of the tip of the reduced outer palea. In other barleys [e.g. Haxtoni in Fig. VI] the glumes of the lateral florets are much shorter in relation to the size of the outer paleae. Inerme may, in fact, be said to possess "long" lateral-floret glumes, whereas most at any rate of the other barleys have short ones. Usually, the outer palea of the lateral floret is small, shrivelled and of "decipiens" type, but, at times, it attains a very considerable size and may even become fertile. In an ordinary growing, a very small proportion of ears is found among whose lateral florets—perhaps in three or four cases per ear—large size or even fertility is achieved. On such an ear the remaining lateral florets are usually quite normal (i.e. they have the usual reduced outer paleae).

"Fluctuation" among lateral florets is considerable in all crosses [cf. Engledow(1)], but it reaches a most acute stage in crosses of which one parent is "Inerme." In order that the difficulties may be realised

and that there may be no misapprehension as to the susceptibility of such investigations to error, an account is given of some of the more extreme cases of fluctuation. Harlan and Hayes(17) have already recorded a case of lateral floret fluctuation. The photographs here published (see plate) need little comment:

- Fig. I { (a) = one ear of an  $F_2$  plant; laterals very reduced.  
 (b) (c) (d) = ears from plants of the  $F_3$  progeny. In (d) the laterals resemble (a), in (b) they are somewhat larger, while in (c) [a late ear from an  $F_3$  plant] many of them are fertile. (a) is a type which usually "breeds true," being in fact the "semi-decipiens" type which is characteristic of "Inerne."
- Fig. IV { (a) and (b) are ears from a single  $F_3$  plant. In (a) median and lateral florets are both awned fully and fertile, while in (b) only the medians are awned and fertile. All the awns in both ears were removed to simplify the photograph. Thus on this plant one ear [(a)] presented the customary *H. hexastichum* appearance, while the other [(b)] simulated a typical *H. distichum*.
- Fig. III { (a) and (b) = ears from a single  $F_3$  plant, (b) being from a very late tiller. Both its awns and its laterals are larger than those of (a).

The examples of fluctuation which have been photographed are, of course, extreme ones; but they indicate the nature of the phenomenon.

As in the case of the awn, so here, classification is a difficulty. The possibility of fluctuation must be borne in mind and the best safeguard against error is a wide experience of the range and nature of fluctuation in parent varieties and in their hybrid progeny. An  $F_3$  family, even if it contains some late "fluctuant" plants, may be classified with fair confidence provided it is composed of not less than about six plants. Very small families are, as always, a danger—and some such families are bound to occur.

#### § VI. VIEWS UPON THE INHERITANCE OF THE LATERAL FLORET IN BARLEY.

Some observations upon this subject have been made in a previous paper [Bibliography(1), pp. 102-108]. Since then has appeared an interesting publication by Harlan and Hayes(17) in which is given evidence of the existence of two factors for the lateral floret [a two-factor difference between a *H. hexastichum* (of the vulgare kind) and a *H. distichum*]. The authors state (p. 578) that "Fertility is a definite, tangible, measurable condition...." They recognise classes of fertility which are characterised by certain percentages of fertile to total lateral florets on the ear, e.g. 0-79 per cent. and 80-100 per cent. in one division and 0 per cent., < 5 per cent. and 5-100 per cent. in another division. In



Fig. I

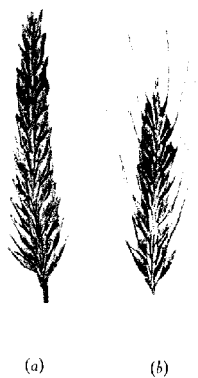


Fig. II

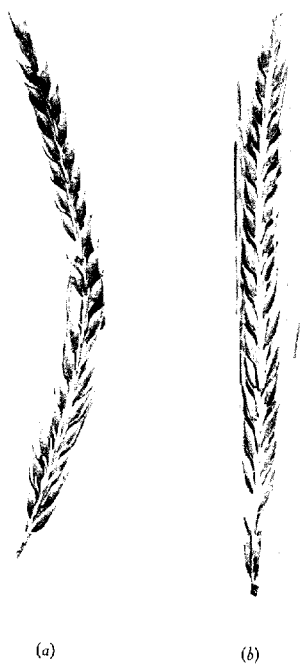


Fig. III



Fig. IV





an English climate Haxtoni (one of their parental forms) and the segregates from its crosses show such extreme fluctuation that percentage-fertility classes would be quite impracticable. It is almost certain that in a large  $F_2$  of a Haxtoni cross there could be found some healthy plants any one of which would have tillers, exhibiting such divergences of percentage lateral fertility as would warrant the inclusion of the plant equally in any of three or even four such classes. The tendency to bring into existence such "mathematical" plant-characters for which there is no *prima facie* biological guarantee, appears to be dangerous. It has often led to elaborate investigation, the result of which has been a negative one [cf. Collins<sup>(18)</sup> and observations upon this reference in § IX, below].

Particularly perhaps in the case of lateral-floret fertility in barley, it seems undesirable to employ artificial "mathematical" characters without first carrying out a statistical investigation to vindicate the biological significance (tantamount perhaps simply to the constancy) of the numerical limits by which such characters are defined.

Despite the closeness of observation to expectation which the authors found, fluctuation was not absent as the following sentence shows: "Group 7 may be considered as composed of plants homozygous for 2-rowed, since with the exception of the two plants noted there was no fertility exhibited in the progeny." The  $F_2$  numbered 87 plants and of these 5 fell into Group 7. It was in the  $F_3$  progeny of one of the 5 (i.e. 20 per cent. of the whole) that the two aberrant plants were found. To demand that there should be no aberrants from a character so full of vagaries as the lateral floret, would seem like demanding the impossible; but the precision of sorting which differentiates between 0 per cent. and < 5 per cent. or between < 5 per cent. and 5-100 per cent. depends for its very existence upon a most strict observance by the plants of the prescribed limits.

#### § VII. SOME EXPERIMENTAL RESULTS UPON THE INHERITANCE OF THE LATERAL FLORET IN BARLEY.

From data already published the conclusion has been derived that the four common types of lateral floret—those which characterise the four main groups, *H. hexastichum*, *H. intermedium*, *H. distichum* and *H. decipiens*—form a series of "multiple allelomorphs" or "alternative allelomorphs." Any two members of the four, when crossed, give an  $F_2$  in which 1 : 2 : 1 segregation is shown. Crosses of members of these four types with *Inerme*, allowed a considerable body of  $F_2$  data to be ob-

tained. This was withheld because wide fluctuation in both awn and lateral floret rendered the raising of  $F_3$ 's imperative. There was ground, too, for supposing that to some extent the fluctuations in these two characters were associated. The  $F_3$ 's—some partial and some complete—have now been raised and the results to be given are based upon them.

GROUP I. *H. HEXASTICHUM* × *INERME*.

*Cross E.30* = *Praecox* × *Inerme*.

It has been explained in § III above that the  $F_2$  of this cross gave a ratio of fully awned to other types of 1 : 3, and that the  $F_3$  families served to classify the  $F_2$  plants thus:

- ( $\alpha$ ) Plants indistinguishable from *Praecox* (the 6-row parent).
- ( $\beta$ ) Plants heterozygous for awn factor.
- ( $\gamma$ ) Plants as awnless as *Inerme* itself.

Moreover ( $\alpha$ ) : ( $\beta$ ) : ( $\gamma$ ) = 1 : 2 : 1.

An endeavour was made to detect different types of lateral in ( $\alpha$ ), ( $\beta$ ) and ( $\gamma$ ) respectively. The results were:

In the  $F_3$  families of type ( $\alpha$ ) all the plants were like *Praecox*, the lateral florets always set plump grains and bore awns. Thus, in this case, the awn and the full fertility of the lateral floret appear to be inseparable. Possibly there were slightly different size-types in the laterals of the plants of this group, but the closest scrutiny failed to effect consistent sorting. It may be that there segregated—quite independently of the presumed awn-cum-fertility character—a purely morphological character which affected the size or shape of the outer palea of the lateral floret.

In the  $F_3$  families of type ( $\beta$ ) were three kinds of plant. The first was apparently identical with the *Praecox* (6-row) parent in both fertility and awn of the laterals; the record was best covered by the general designation "half-awned." This record type displayed a very big range of lateral floret forms, from "some florets of the ear fertile" to "all florets of the ear exactly like the 'normal' *Inerme*." Differences among the lateral florets of the same ear were in some cases extreme. The third kind—awnless save for the fluctuations which characterise *Inerme* itself—had in nearly all cases the "normal" *Inerme* lateral; but in a very few instances a fertile (grain setting) floret was seen, its companions upon the ear being "*Inerme*" in form. Thus it may be said that in this cross the absence of awn and the *Inerme*-lateral are apparently inseparable.

In the  $F_3$  families of type ( $\gamma$ ) some plants had most of their lateral

florets fertile, some had rather inflated but non-fertile laterals, some had all their laterals of normal *Inerme* form, and some had one (or two) fertile with the rest like the parental *Inerme*. The range of lateral-types was therefore greater than that shown by the "awnless" plants of the ( $\beta$ ) families. It is difficult to suggest an interpretation of these results. To have attempted a classification based upon percentage of fertile laterals per ear would have led to no useful result for there was a whole range of sizes of non-fertiles. Perhaps the simplest explanation—purely hypothetical—is the one that, as sorting proceeds, thrusts itself upon the attention, viz. that the lateral florets of "*Inerme*" type are potentially fertile but that some morphological or possibly physiological condition presents the manifestation of the potentiality. This condition must, of course, be presumed to be linked with awnlessness and it must further be assumed that in the cases of certain florets the inhibiting condition is imperfectly developed so that these florets are able to set grains. Possibly two such inhibiting factors are involved. They may segregate independently and the awnless plants [members of ( $\gamma$ ) families] which have a considerable proportion of their laterals fertile may have some specific genetic constitution in regard to the second of these hypothetical factors. Speculation such as this is, of course, rather primitive and not at all likely to lead to a furtherance of real knowledge; but it is the only form of speculation which is at present applicable despite the fact that a complete  $F_3$  has been raised (containing some 4000 plants). Even were the formidable task of a complete  $F_4$  undertaken, the hope of finality would still be very small. Here, as in the case of many other "eye-characters," a simple lesson is plainly pointed. The raising of successive generations and sorting by eye should be discontinued until the "eye-character" can be displaced by some positively observable character which, whatever its outward significance, has a real significance in the make up or functioning of the living plant.

A point of some interest remains. In all the crosses recorded in § III above, save one, there is a defect of the fully awned type as compared with 1 : 3 expectation. As already explained the defect is always too small to be statistically significant, but it may imply a very high coupling. If fertility of laterals (regular fertility as in *Praccox*) be not genetically inseparable from, but simply highly coupled with, full awn, a small percentage of fully-awned 2-row plants should appear. Such plants have never appeared in the  $F_2$ 's of 6-row  $\times$  *Inerme* crosses, which have so far been bred, but in the  $F_3$  of cross *E.30*, three such plants have been observed. They have typical distichum laterals and very lax

ears. So few in number (3 in 4000), so lax in rachis, and so completely unexpected (the type has long been sought in similar  $F_2$ 's and  $F_3$ 's), they arouse fears of an error (although past experience gives every confidence) in the methods of sowing, etc., employed. The full data of the cross, the existence of these "awned 2-rows," etc., has been recorded here because it is felt that a repetition of the cross on the lines already followed cannot lead to more precise results. To guard against possible errors and to allow an opportunity for the re-appearance of this rare type it is intended to raise an  $F_4$  of 2000 families from the seeds set by plants of the ( $\beta$ ) families.

Cross E.28 = Parallelum  $\times$  Inerme.

This cross has a general similarity to E.30 (just described) except that Parallelum has a denser rachis than Praecox. In the  $F_2$  it was impossible to effect a consistent sorting of lateral floret classes and only a partial  $F_3$  was raised (plant-breeding selections only). An interesting feature of the  $F_3$  was that in the families bred from  $F_2$  plants homozygous for "awnlessness," the laterals were all of "normal" Inerme type. This contrasts rather markedly, of course, with the case of Cross E.30, where deviations from the "normal" Inerme were found in corresponding families. Segregation of "density of rachis" evidently occurred and, in particular in awnless forms, rather curious shapes of ear resulted. Two or even three factors may have been involved in the production of these degrees of "rachis-density" (or differently regarded, of "ear-shape"), but to have sought to establish their existence would have been to add one more series of difficult "eye-judgments," and the endeavour was abandoned early in the investigation.

The form of cross considered in this group—*H. hexastichum*  $\times$  *H. distichum* (in the above crosses, *H. d. Inerme*), has been made many times by a number of investigators. Until Harlan and Hayes<sup>(17)</sup> published their results it was accepted as a fact that the *H. intermedium* form did not appear as a homozygote in the  $F_2$ 's of such crosses. These two authors found that in some cases the homozygote *H. intermedium* did appear in the  $F_2$  of the Hexastichum  $\times$  Distichum cross; in others it did not. They consequently postulated *inter se* differences of the Hexastichum forms used as parents in the crosses.

Surprise is expressed that neither von Tschermak nor Biffen found homozygous *H. intermedium* forms in the  $F_2$ 's of any of their numerous crosses of *H. hexastichum*  $\times$  *H. distichum*. It may well be that the very great fluctuations of the lateral florets allowed the homozygous "inter-

mediums" (if such did occur) to escape observation. Some crosses belonging to the same category have been described in a previous publication [Engledow (1), p. 100]. One of them, viz.  $E.43 = \text{Praecox} \times \text{Archer}$ , was grown to a complete  $F_3$  in 1920 (over 500 families containing about 5000 plants). The degree of lateral floret fluctuation was so extreme that no consistent result was obtainable; but certainly no  $F_3$  family occurred from which the conclusion was to be drawn that there had been  $F_2$  plants homozygous for the characteristics of *H. intermedium*. That the further raising of  $F_2$ 's and  $F_3$ 's should be deferred until a better understanding of "fertility" of lateral florets has been gained, seems once more to be clearly implied.

GROUP II *H. INTERMEDIUM*  $\times$  *INERME*.

Cross  $E.37 = H. \text{intermedium}$  (var. *Haxtoni*)  $\times$  *Inerme*.

The  $F_1$  plants had, in some cases, a small proportion of fertile laterals (i.e. with grains set); in others of the same year, all the laterals failed to set grains but were more inflated and more pointed than *H. distichum* laterals; there was a considerable range of fluctuation. From  $F_2$  no evidence for lateral-floret inheritance was derived.  $F_3$  was raised complete and was classified as already described [§ III, Group II, above] on an awn basis into groups of families, viz.: ( $\alpha$ ) with all plants fully awned; ( $\beta$ ) "splitting" for awns; and ( $\gamma$ ) with all plants as awnless as *Inerme*. The result was ( $\alpha$ ) : ( $\beta$ ) : ( $\gamma$ ) = 1 : 2 : 1.

The next step was to examine ( $\alpha$ ), ( $\beta$ ) and ( $\gamma$ ) one by one for types of lateral floret. It will be recalled that the glumes of *Inerme* are relatively long while those of *Haxtoni* are relatively short [see § V, above, and Figs. V and VI], and the first fact disclosed by the examination was that extent of awn and relative size of glume were associated. Full-awn forms<sup>1</sup>, i.e. plants of the  $F_3$  families constituting class ( $\alpha$ ) had the relatively short glumes of the *Haxtoni* parent; in class ( $\gamma$ ), where the awn was absent, the relatively long glumes of *Inerme* were found; while in ( $\beta$ ), in addition to plants resembling ( $\alpha$ ) and ( $\gamma$ ), were "half-awned" forms with glumes relatively of "intermediate" length. No measurements of glume-length were made and the observed "association" of awn and glume-length rests solely upon eye-judgment.

Once this "association" had been confirmed it was possible to devote attention to the evidence of segregation in other lateral floret characters. The time-honoured standards of "fertility," as judged by the eye, were employed. These standards are necessarily broad and elaborate—they

<sup>1</sup> The awn of the outer palea of the median floret is the one implied of course.

must allow for the known facts of fluctuation, such as one fertile lateral on an ear which is otherwise absolutely "normal" Inerme in its laterals. In fact, as in all such cases, the standards are almost intuitive; they represent an intuition arising from extended observation. Fluctuation is so prevalent in work of this kind and so little understood that eye-judgment cannot conscientiously be described as reliable. It is with this proviso that numerical results are set forth (Tables XI and XII, below). The  $F_3$  families of awn-group ( $\alpha$ ) [and similarly of ( $\beta$ ) and ( $\gamma$ )] were placed in three classes thus:

I. All plants with laterals as fertile as those of Haxtoni.

II. The plants of three types, viz. Haxtoni, Inerme and an "intermediate."

III. All plants with laterals of the type of Inerme (i.e. normally very reduced but now and then one or two small grains set on an ear).

Attention must be called to another small matter before the results of the classification are detailed. Although classes I, II and III could be made in both ( $\alpha$ ) = full-awn and ( $\gamma$ ) = awnless, the reduction of the outer palea of III was less marked in ( $\alpha$ ) than in ( $\gamma$ ). It seemed, in fact, that just as awns seem to be associated with relatively short glumes, so they are associated with a tendency to a rather large outer palea in the lateral floret [independently of fertility in the floret]. Figs. (text) VII-IX illustrate the types found in this cross and from them the "associations" of characters here described are apparent.

The numerical results are given in Tables XI and XII, in which, as in previous tables, the letters *A, B, C, ...* designate individual  $F_1$  plants or their progeny, according to the context.

Table XI.  $F_3$  families of Cross E.37.

F <sub>1</sub> progenitors	Awn-types									Total
	(α)			(β)			(γ)			
	Lateral florets			Lateral florets			Lateral florets			
	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	
E.37.A	5	10	4	4	18	14	4	13	8	80
E.37.B	6	8	4	18	25	10	9	11	9	100
E.37.C	9	19	7	28	32	18	5	22	7	147
E.37.D	4	6	5	9	14	10	4	6	4	62
E.37.E	3	7	1	4	11	5	2	2	2	37
E.37.F	1	4	5	7	14	6	2	3	2	44
E.37.G	4	18	3	5	22	9	8	14	5	88
Total	32	72	29	75	136	72	34	71	37	
Total	133			283			142			558

The frequencies in the table are those of  $F_3$  families and, from what has been said above, it will be observed that they may equally be treated as the frequencies of the  $F_2$  plants from whose seed the  $F_3$  was raised. Table XII sets forth the errors of observation, etc.

Table XII. *Résumé of Table XI with errors, etc.*

	(α)			(β)			(γ)			Total
	(I)	(II)	(III)	(I)	(II)	(III)	(I)	(II)	(III)	
Observed numbers ...	32	72	29	75	136	72	34	71	37	558
Expected numbers (1:2:1 basis) ...	33.25	66.50	33.25	70.75	141.50	70.75	35.50	71.00	35.50	558
Observed percentages	24.06	54.13	21.81	26.51	48.05	25.49	23.95	50.00	26.05	—
Expected percentages	25	50	25	25	50	25	25	50	25	—
Actual errors (%) ...	0.94	4.13	3.19	1.51	1.95	0.49	1.05	0.00	1.05	
Standard error of ex- pectation* ...	3.75	4.33	3.75	2.57	2.97	2.57	3.63	4.19	3.63	

\* See footnote on p. 166 (above).

Observation may be called fairly close to expectation on the 1:2:1 basis.

For the cross *H. intermedium* (var. Haxtoni) × *Inerme* the following conclusions are therefore indicated:

(i) Relatively short glumes and a relatively large outer palea in the lateral floret, are associated with a full awn in the median floret and conversely.

(ii) "Fertility" of the lateral floret segregates on a 1:2:1 basis.

(iii) Sorting into fertility classes is made possible only by making allowance for fluctuations, such as observation has shown to occur in the parent varieties.

The genetic constitution of *H. intermedium* (var. Haxtoni) must consequently be supposed to be radically different from that of *H. hexastichum* (for var. *Praecox* and var. *Parallelum* at any rate) for in the latter form "fertility" of lateral florets was shown to be either quite inseparable from the full awn or coupled (linked) with it to a very high degree.

#### GROUP III. *H. DISTICHUM* (AWNED) × *INERME*.

For this group no detailed observations upon lateral florets are available. Partial  $F_3$ 's were raised in four crosses, the *Distichum* (awned) parents of which were:

For Cross E. 32.	Russian Chevallier No. 7	—ear very lax (narrow)
E. 34.	Russian Goldthorpe No. 9	—ear very dense (broad)
E. 35.	Russian Goldthorpe No. 10	—ear very dense (broad)
E. 41.	Plumage	—ear very dense (broad)



From the complete  $F_2$ 's and the partial (plant-breeding)  $F_3$ 's the following facts have become clear:

(i) The lateral florets of fully-awned plants are never reduced like those of *Inerme*. In Cross *E.32* little range of size was shown in the fully-awned extracts, but in the other crosses (the awned parents of which were broad-eared) distinct differences appeared. Density of ear clearly segregated, and the denser the ear the larger the lateral florets. Plants whose ears were as dense as those of the awned-distichum parent had laterals of parental size; those with lax (narrow) ears had laterals of the same size and of the same general appearance as in the "narrow-eared" 2-row barleys such as *Chevallier* and *Archer*, etc.

One homozygously-awned  $F_3$  family of *E.32* was unique in that two of its plants had about 20 per cent. of their lateral florets fertile. The significance of these plants must remain an open question. Nowhere else in Group III ( $F_2$ 's or  $F_3$ 's) were there found awned plants with any fertile laterals.

(ii) Among the plants belonging to homozygously awnless families the lateral florets were always of *Inerme* type, i.e. reduced (semi-deciapiens) but with one or two becoming fertile per ear in a very small percentage of the total number of plants (exactly as may happen in the *Inerme* parental form itself).

(iii) Half-awned plants showed a wide range of lateral-floret forms—never quite as reduced as in normal *Inerme* and rarely as large as in the awned parent.

(iv) Relatively long glumes—as in Group I and II—appear to be associated with the *Inerme* type of lateral and conversely.

*Conclusions.* (i)–(iv) show a general agreement with those derived from cross *E.37* (described under Group II above) except that, of course, they do not bear upon the question of the fertility, but only on that of the morphology of the lateral floret. The fact that glume, outer palea of the lateral floret, and awn of the median floret appear to behave as if governed by one and the same genetic factor is interesting. It does not, however, furnish a very helpful means of checking the form and fluctuation of any one of the characters by observation of the other two. For this, some much more profound botanical inter-relationship will have to be discovered.

§ VIII. SOME GENERAL CONCLUSIONS UPON THE INHERITANCE  
OF THE LATERAL FLORET IN BARLEY.

The preceding paragraph is heavily loaded with close descriptions of anatomical features. This is inevitable for the elusiveness of the character has led to the observation of every other character which might be of assistance. Shorn of side issues the main conclusions are that "fertility" of the lateral floret segregates on a 1 : 2 : 1 basis. In *H. hexastichum* crosses it is inseparable from "full awn" whereas in *H. intermedium* crosses this is not the case. Relatively long glumes are inseparable from "awnlessness" and conversely; while, finally, dense-rachis is similarly inseparable from a large outer palea (lateral floret).

Setting aside as a "fluctuation" the occasional but rather striking fertility of isolated laterals on otherwise Inerme ears, there yet remain complications. In § VII (Group I) the possibility of a high coupling (linkage) between "awn" and "fertile lateral" has been indicated; the two plants showing some fertile laterals in an  $F_2$  derived from *E.32* (Chevallier  $\times$  Inerme) are quite beyond explanation at present; and, though not connected with Inerme crosses, the bi-factorial effects alleged by v. Ubisch<sup>(10)</sup> and the production of *H. intermedium* in a hitherto unrecorded manner by Harlan and Hayes<sup>(17)</sup>, must be added to the list of lateral floret "complications." The suggestion that the four commonly recognised forms of lateral floret constitute a series of multiple (alternative) allelomorphs, while giving no assistance in the solution of the general problem, adds to its interest.

By some means, the problem must be solved and the future line of attack is a debateable question. Repetition of the methods which have been carried on for fifteen years by several independent investigators is certainly a last resort—but it is no more. "Fluctuation" has been the bar to success of the method in the past and must always so remain. To ascertain the cause of the fluctuations—the reasons for the observed responses to environmental differences—must be the first step; and this implies a knowledge of the exact histology of the various forms of lateral floret and of the physiological factors by which they are affected. Few, if any, of the floral arrangements in other plants are comparable to e.g. the ordinary distichum barleys in which, with unfailing regularity, six rows of florets come into existence, but two only, and these two placed in an unvarying position on the rachis, are able to set grains. No one has yet determined at what stage in the life cycle these laterals fail. Possibly their failure is due to no fault of stamens or ovaries. It

may be that the paleae are too small to afford room for the full development and functioning of the reproductive organs. Rough hand-sections of the rachis appear to indicate the possibility that there may be a deficient vascular supply to the lateral florets in the various 2-row barleys. A third possibility is that the laterals develop later (chronologically) than the medians of the same ear. Usually, the medians effect self-pollination, while still protected from wind, sun, rain, etc., by the leaf-sheath, so that at the time of emergence of the ear from the sheath the laterals (if later in development) may not yet be sufficiently mature to allow of self-pollination. Lacking the sheltered conditions under which the medians effect self-pollination, the laterals may in this way have infertility forced upon them. This third explanation is not likely to apply to the ordinary "distichum" barleys such as Archer, etc., but may possibly be correct in the case of forms which, like *Inerne*, have "occasional" fertile laterals. Anthony and Harlan<sup>(20)</sup> have conceived the idea of classifying lateral florets by the germinability of their pollen (in the laboratory), but have not yet attained decisive results.

These questions are some of a number which point out untried lines of investigation. Fifteen years covered by the close observations of a number of workers have led to discordant views upon the genetic constitutions of lateral florets and one is forced to remark that an attack along a new line seems to be essential. The work that has been done has served to reveal a fascinating genetic problem, but it would be regrettable if a corresponding amount of endeavour were squandered in unpromising repetition instead of being diverted to the more hopeful but neglected line of physiological-anatomy. It is not suggested that barley-laterals are unique in requiring this change of direction of genetic inquiry; the change would be beneficial in connection with many plant-characters—in particular the "multifactorial" characters—but perhaps the need is here emphasised with uncommon clearness by the history and results of past investigation.

#### § IX. SOME CONSIDERATIONS ARISING OUT OF THE FOREGOING PARAGRAPHS.

A few brief sentences suffice to describe the essence of the experimental results contained in the foregoing paragraphs. Some new 1 : 2 : 1 ratios have been established and their simplicity is out of concord with certain earlier findings of similar investigations. Fluctuation has been proved to be a serious obstacle, its limits of range have been explored, but its causes and significance have remained a mystery. In fact, the

investigations—both those recorded here for the first time and those published earlier and here reviewed—have yielded a tangled mass of observations from which may be gleaned certain interesting genetic conclusions. The conclusions are—in the main—genetic ratios, ratios which testify, as did Mendel's first ratios in 1865, to the occurrence of an ordered "segregation" of inheritance factors. This review of experimental results applies in full to many other of the numerous series of genetic records which have been published since 1900 but it is, perhaps, particularly applicable to the awns and lateral florets of barley for these are among the best known and best tried of the "genetic" plant-characters.

It is impossible, after weighing these facts, to evade the questions "What is the value of a continuance of this repeated establishment of 3 : 1 and other ratios?" and "Is sufficient attention being given to the raising of a superstructure on the foundation constituted by these ratios?"

At the outset, it must be made clear that these questions are looked upon as in no need of an answer in the case of those lines of investigation which relate to "Reduplication" (Linkage), "Gametic Contamination," "Multiple Allelomorphs" and all kindred phenomena, for investigations such as these have a definite goal beyond the mere establishment of "ratios." Much effort continues to be devoted, however, to experiments which terminate—and many experiments do so terminate—in further "ratios" and in nothing more. It is in connection with this kind of experiment that the above questions seem thoroughly pertinent.

There is, undoubtedly, a growing tendency to select as "experimental characters" those which are of some economic importance and in attempting to vindicate repeated "ratio" experiments it is best, perhaps, to consider first the economic value that such experiments have had in the past and their possible future worth. More than a century before Mendel made his first cross, Robert Bakewell of Dishley had begun to improve British live-stock by carefully regulated breeding. Plant-hybridisation, though it remained for so long the speculative study of men such as Kölreuter, Knight, Herbert, v. Gaertner and Noudin was definitely and successfully brought to the assistance of Agriculture by Farrar in Australia and by the brothers John and Robert Garton in England. These last-named commenced their hybridisation work in 1880 and from that time onward they and their colleagues (at the present time Garton's Ltd., of Warrington) have continued to put new cereal varieties on to the market. A popular account<sup>(19)</sup> of plant-

hybridisation, written in 1900, explains the procedure of the raising of new varieties in vogue in the year which brought Mendel's discovery out of obscurity. To employ a great number of widely different parents in the making of crosses and by eye-judgment and trial to select the most valuable plants of the progeny—this was the method then in use and, when the bare fact is exposed, it is the one still in use. Although eye-selection remains the prime agent, there has been a very marked change in outlook. Twenty or so years ago there prevailed an unaccountable optimism which held that if far-removed forms—the further the removal the better—were crossed in a great number of different combinations, then “discoveries,” “the ideal yielder,” the “ideal disease-resister” and so on *must* be made. Such would appear as the treasures among a huge dross of “types.” The advent of the scientific plant-breeder cast away this baseless optimism and in its place installed the principle that parents must be selected for their known specific values. For example, it was argued that to produce a “strong” wheat from a cross, it was essential to use “Fife” as one parent. What genuine “improvements” have been produced in this country must all be placed to the credit of this general principle—a principle which comes as a natural one to plant-breeders now. It will, however, be readily apparent that the introduction of this principle is a contribution rather of insight and clear thinking, than of experiment. Of what value then, from the economic standpoint, has been this repeated finding of ratios? The whole value is expressed by the single word “segregation.” When it became known that there were regularly segregating “unit characters,” plant-breeding received an immense impetus and to this impetus may be traced some of the best of the cereal varieties now in existence. The conception of “independent” (unit) characters removed the former fear of the “reversion” of hybrids to the inferior parental types and restored the long-lost hope of the possibility of “fixity” of type. In so far as it has confirmed Mendel's theory and established its applicability to agricultural plants, “ratio-finding” investigation has been of inestimable value. To-day, however, there is no doubt as to the general validity of the theory and therefore “ratio-finding” must seek some other economic justification. [Perhaps the safer form of statement is that Mendelism has a general validity which the coarse method of the ratio-determination of eye-characters is not likely to be able to challenge.]

Those who, since 1900, have raised new and better cereal and other varieties have, of course, consciously depended in their investigations upon the fact of segregation. Further than this, they have, in many

cases, conducted ratio-experiments in the early stages of the work which has had its fulfilment in the production of valuable new strains. It is, however, a most debateable question, whether they could not and would not, have produced precisely the same final result, if their academic interest had not led them to include a "ratio-determination" as part of their procedure.

The past indebtedness of plant-breeders to Mendelism is unquestioned; exactly how it was incurred is to a great extent a matter of opinion; and interest must now centre on the possible future value of simple "ratio-Mendelism."

In the cereals, at any rate, it will be of no economic advantage to establish further ratios in connection with simple, eye-judged, morphological characters. Many such are already on record and, except as confirmations of the very general theory of Mendelism, they have not been an aid to the plant-breeder. To discard such characters for those of direct practical importance seems the first step. The step has already been taken in connection with cotton, wheat, barley, oats, maize, potatoes, etc., but the achievements have been few. As long as the eye is allowed to determine the form of Nature's "hereditary-units" it will remain a just contention that the results achieved in work of this kind could equally well be achieved without calling upon mathematics to reinforce eye-judgment by "ratios" derived from eye-observation. Apart from this, is the fact that the economic features of prime importance—such as Yield and Standing Power in the cereals—are far more complex than awns and fertile lateral florets, etc. It is not likely to be of either academic or economic interest to know whether the eye-sorting of an  $F_2$  on the basis of some morphological character requires two hypothetical factors to explain the observed ratio, or only one. In any case, the "eye-character" is only a manifestation and one depending in part for its precise form upon environment. Upon what else does it depend? The customary answer is, upon a "factor" or upon two or more "factors." Let this answer be accepted and let it further be accepted—as thanks to Morgan and his collaborators it now well may—that the factor is a "something" in a chromosome. That a "factor" exists and segregates is a helpful idea and a very well tried one, but in what manner does the factor operate? Its presence is responsible for a potentiality, the potentiality controlled by growth-environment produces a certain manifestation—but what is the exact physiological nature of the potentiality? This seems to be the enquiry upon which genetic effort—forsaking the simple ratio-quest—should now concen-

trate. If, in the case of a few readily recognisable eye-characters, the causative physiological potentialities could be exposed, it might be possible to undertake an investigation of the inheritance of such highly important attributes as "yield" with fair prospect of success. Up till now researches on yield-inheritance have employed only the unwieldy standards of physical and numerical plant attributes. Before any progress is made resort will have to be had, it is felt, to the fundamentals, the determining physiological potentialities, and these elusive features may, perhaps, best be sought by first making acquaintance with them in the simple eye-characters of plants the inheritance phenomena of which have been already investigated. A knowledge of the potentialities which lie behind the manifestations (for convenience called the plant-characters) represents the "superstructure" which, logically, ought to be building upon the foundation of the ratio. It is, perhaps, a justifiable comment, that the foundations are being monotonously extended while the superstructure suffers neglect.

As a first step in the endeavour to elucidate "potentialities," suitable forms of "character" will have to be decided upon. It is of interest briefly to trace the development of the choice of "characters" in genetic work. For a time the simple eye-judged characters such as "tall and short," "rough and smooth," etc., were alone observed. Measurements—first linear, then superficial, then of weight, etc.—came next. Chronologically, perhaps, the series was then extended by the "characters" implied by "multiple factors." A sub-extension was introduced by the still more highly imaginary "characters" demanded by the supposed existence of a great number of "minor multiplying factors." Ratios of various dimensions came to be adopted for the reason that, wisely chosen, they might help to eliminate the disturbances for which environmental differences are usually responsible in absolute-measurement characters (such as length of rachis, etc.). A novel and highly artificial character—best referred to, perhaps, as a "mathematical" character—has not infrequently been used. Collins<sup>(18)</sup> in one investigation sought the aid of this conception of a character because he found that in maize the facile recognition of "types" was not at all easy to achieve. The general argument was this. If, in some selected pure line population, every possible "character" of the plant be investigated—purely botanical and economic, eye-obvious and eye-elusive alike—and if correlations be found between certain economic characters and certain non-economic but readily observable ones, then the economic characters will be traceable in hybridising and selection by observation of the correlated ones. To

measure correlations, it was necessary to give numerical representation to all the experimental characters. One example of the procedure must serve for illustration. "Hairy"ness" was one of eleven selected characters and it was measured by:

- (i) The number of nodes per plant with completely hairy sheaths.
- (ii) The number of nodes per plant with a complete circle of hairs at the throat of the sheath.
- (iii) The length of the longest tuberculated hairs.

There is, perhaps, little room for surprise that an elaborate investigation of such characters as these led to a negative result—there was, in maize, a marked absence of "genetic correlations."

This brief survey of the history of development of the conception of plant-characters, has one very striking feature. There has been a steady departure from the ideas of character in which the systematic botanists used to trade—and still trade—and, bit by bit, very artificial, "mathematical," conceptions innocent of any biological basis, have come into vogue. It seems most improbable that the observation of characters of this kind—although their possible number is indefinite—will ever lead to a knowledge of physiological potentialities.

There remains an outline-consideration of the steps to be taken to glean a knowledge of physiological potentiality. To a great extent, the nature of the experimental "character" must direct these steps. For the awn and the lateral floret of the barleys four possibilities are readily apparent, viz.:

( $\alpha$ ) A search may be made for other eye-characters that seem to be modified and to fluctuate *pari passu* with the awn or lateral floret. It has, for example, been suggested (§ VIII above) that the vascular bundle supply is a "co-variant" with lateral floret-form. Possibly it may prove to be causative rather than co-variant. If a group of co-variants can be found, the features they possess in common may point to some physiological activity as the "potentiality" of which they are, in part at any rate, manifestations.

( $\beta$ ) The botanical homology of the character may be important in indicating from what source to seek co-variants and of what nature the governing potentiality is likely to be. Paragraph (iv) (above) contains evidence that the accepted view of the homology of the awn needs examination.

( $\gamma$ ) In all cases a histological study of development is likely to be important. For the lateral floret it is certain to be indispensable. The difference of the developments which in *H. hexastichum* lead finally to



six rows of fertile florets and in *H. distichum* to two rows of fertiles and four of infertiles, offers a fascinating study and one that will have to be undertaken before the significance of the manifold floret forms can be properly assessed.

(δ) The growing of experimental plants under a wide range of controlled environments may, perhaps, lead to a knowledge of the possible limits of "fluctuation." It may indicate the extent to which the different "manifestations," which are called varietal and sub-varietal "characters," can overlap. A knowledge of the bounds outside of which a manifestation cannot extend may cast light upon the nature of the potentiality of which that manifestation is an expression. Some of the  $F_2$  "Inerme" extracts in which very occasional fertile lateral florets may appear (say one or two per ear) seem likely to be suitable material for work of this kind.

The present-day is indebted for its knowledge of Mendelism, in a measure almost too great for acknowledgement, to the "ratio-finding" experiments of the past; but the time seems now to have come when investigation should be raised to a new plane, the plane upon which physiological potentiality is, in place of eye-obvious manifestation, the acceptance of the phrase "the character of the plant."

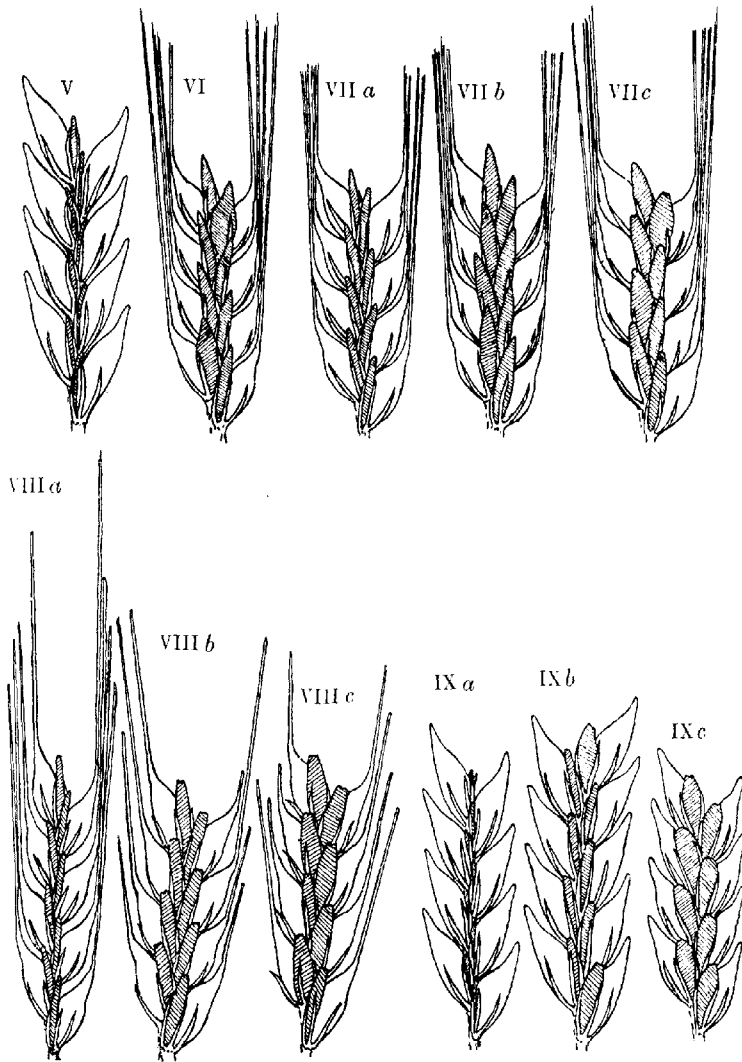
Fig. V. *Inerme* parent.Fig. VI. *Haxtoni* parent (tops of awns not shown).

Fig. VII. Fully awned types.

(a) Laterals not fertile; glumes short; outer paleae of laterals not extremely reduced.

(b) Laterals of intermediate size and usually fertile.

(c) Laterals all fertile though irregular in size.

Fig. VIII. "Half-awned" types. (a), (b), (c) as in Fig. VII.

Fig. IX. Absolutely awnless forms. Here in (a) a more complete state of reduction of the outer palea of the lateral floret occurs than in the fully awned forms [cf. Fig. VII (a)]. In (b) two fertile laterals occur with the remainder infertile.

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# A CONTRIBUTION TO THE INVESTIGATION INTO THE RESULTS OF PARTIAL STERILISATION OF THE SOIL BY HEAT.

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THE discovery that partial sterilisation by heat is capable of greatly increasing the fertility of the soil<sup>1</sup>, opens up possibilities full of interest both to agriculturist and to horticulturist. Steaming the soil has already become a common practice by growers of tomatoes under glass, where the soil in the houses when "sick," that is, infertile no matter how heavily manured, is treated with steam, whereby its fertility is completely restored.

Having made some rough outdoor experiments with steam on small plots, and finding that the results were eminently satisfactory, and that further, all weed seeds were completely destroyed, the writer endeavoured to attack the problem of applying partial sterilisation on a practical scale.

In doing so, one of the first questions presenting itself, upon which no information was available, appeared to be the depth to which it was necessary to apply the heat. It seemed obvious that without such information, no estimate could be arrived at as to the cost of the process, nor the best method designed of carrying it out on a large scale out of doors.

A series of experiments was accordingly carried out at Pyrford, near Woking, with a view to ascertaining:

- (1) Whether the effect of heating proved of greater benefit to the soil at any particular level.
- (2) Whether this optimum depth showed variation depending on the season of the year.

The experiments were carried out as follows. A trench was opened in the ground to a depth of 18 inches, against one face of which were placed two upright pegs, having protruding nails in them two inches apart. Across these nails a board was placed horizontally. The straight

<sup>1</sup> *The effect of Partial Sterilisation of Soil on the Production of Plant Food*, E. J. Russell and H. B. Hutchinson.

edge of the board gave the required level, and by this means the soil was removed in layers two inches thick. The soil from each of the nine layers, after being sifted, was placed in two small wooden boxes, measuring  $7 \times 7 \times 2$  inches, each holding 4 lbs. of soil. One of each pair of boxes was then steamed, the other being kept as a control. The steaming was carried out in the following manner. A "boilerette" or water-jacketed boiler as used for cooking purposes was procured, into which three boxes fitted exactly, and in which the boxes were left for one hour after all the soil in them had reached a temperature of close upon  $100^{\circ}\text{C}$ . After removal the boxes were left for a week, when both they and the controls were sown with mustard seed.

Seed was thickly sown, but after germination, when the second pair of leaves became visible, the plants were reduced to twelve in each box. Immediately after the plants came into flower, they were cut off at the soil level, and the total dry weight ascertained to  $\cdot 001$  gram. In summer they were grown in a cold frame or greenhouse, and in winter in a house the temperature in which varied from  $7^{\circ}$  to  $21^{\circ}\text{C}$ . according to the weather. Each experiment thus consisted of 18 boxes, two filled with soil from each layer, one of which was steamed and one a control.

The whole process was repeated in an exactly similar manner at intervals of three weeks. In every case, before each experiment was commenced, the soil was removed from the face of the trench in order to open up a new surface not previously exposed to the air.

Two kinds of soil were used with which parallel experiments were conducted. In one case, soil from a recently made kitchen garden was chosen, which had been trenched to a depth of 18 inches five years previously, and well manured with farmyard manure, but no doubt not always to the same depth. As it appeared possible that such previous treatment might tend to obscure the results, it was later thought advisable to make an identical set of experiments using the soil of a field close by, which had never been trenched, but which had been manured five years previously and laid down to grass. The turf was, of course, removed before this soil was taken.

The attached diagrams illustrate the results from these experiments. They numbered 22 of each soil, and were made, in the case of the garden soil, between April 1917 and August 1918, and in the case of the field soil between August 1917 and April 1919. The time taken for each crop to reach the flowering stage varied from 36 days in summer to 118 in the winter months. Fig. 1 illustrates the great increase of crop obtained by steaming the soil. In this case, the total of the crops which were obtained

in the 22 experiments from each of the nine depths of soil has been taken, the steamed and the unsteamed, and the difference between them expressed as a percentage increase, shown as a curve. Figures, showing the

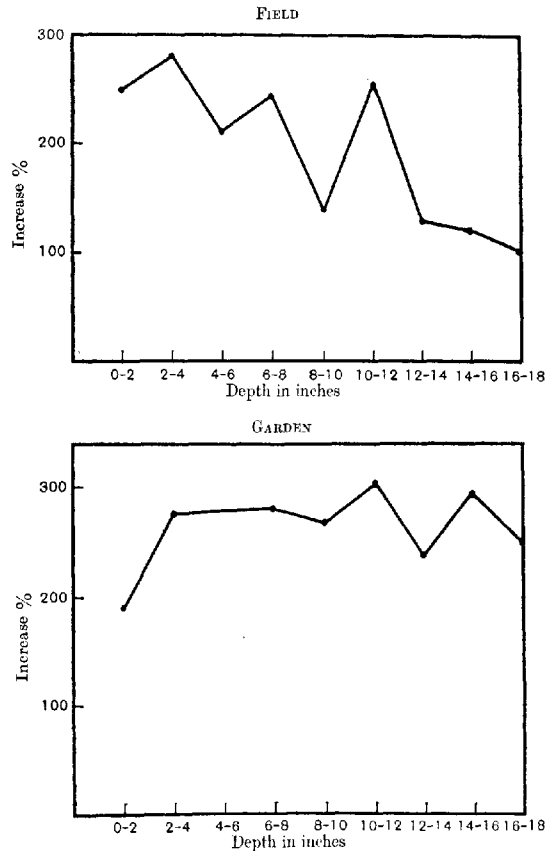


Fig. 1. Curves showing percentage of crop increase on steamed over unsteamed soil at varying depths. Crops from 22 separate experiments in soil from Garden and Field. actual crop obtained from each of the 792 boxes in the whole series, are given in the Appendix.

In these individual boxes there will be observed one instance in the garden soil series (Exp. 1 Garden Soil) where the control box gave a crop

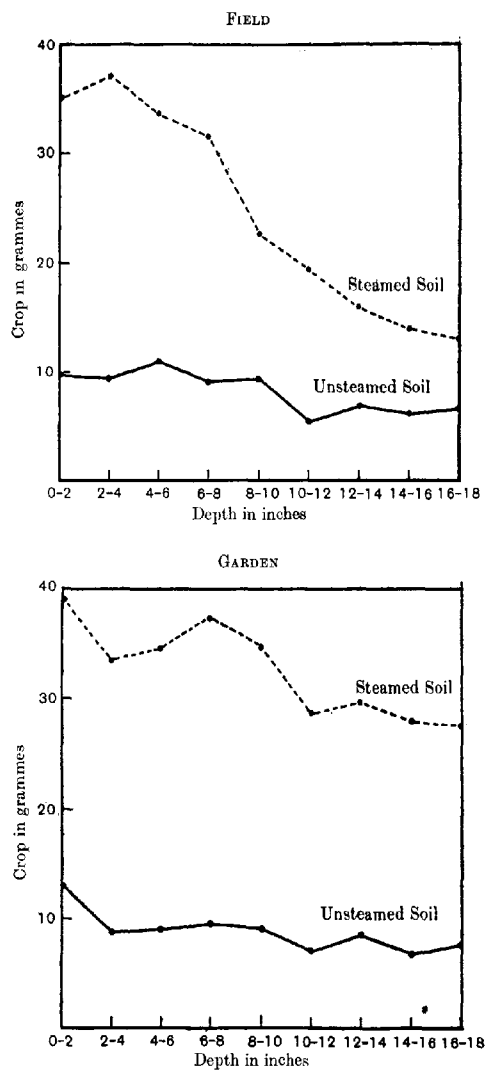


Fig. 2. Curves showing total crop obtained from steamed and unsteamed soil at varying depths. Crops obtained from 22 separate experiments in soil from garden and field.

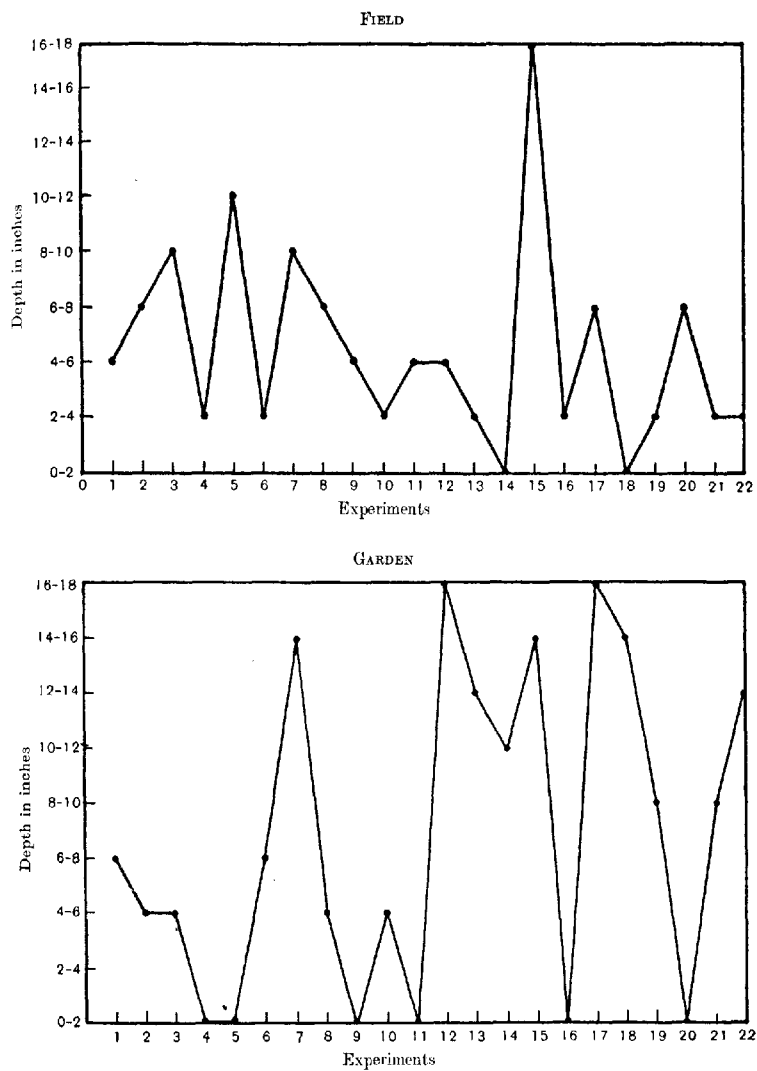
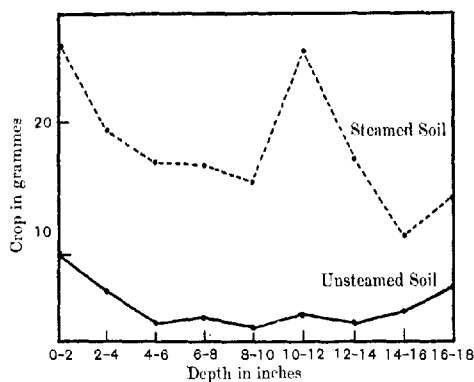
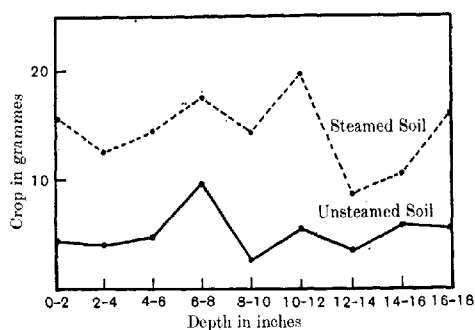


Fig. 3. Curves showing at which depth the maximum increase in each of the 22 series of experiments was obtained.

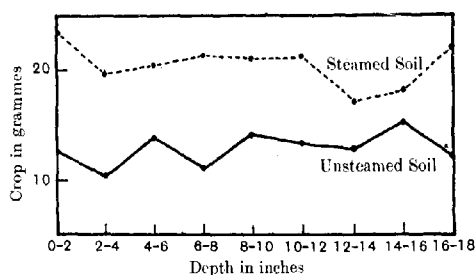




A. Sown Jan. 11th, cropped April 8th.

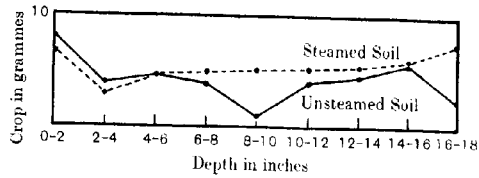


B. Sown April 10th, cropped May 25th.

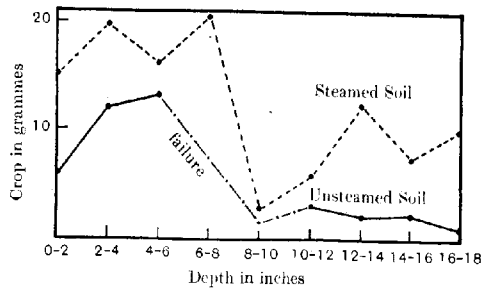


C. Steamed again, sown June 10th, cropped July 15th.

Fig. 4. Five crops grown successively in the same soil.



D. Sown July 16th, cropped Sept. 15th.



E. Steamed again, sown Sept. 24th, cropped April 6th.

Fig. 4 (continued).

very much larger than that which had been steamed. The writer is inclined to think that the two boxes may have been accidentally changed over during the course of the experiment as it never occurred again in the garden soil experiments. As will be seen, individual boxes of steamed soil in some of the experiments with field soil failed to show an increase over the controls, generally at the lower depths, but in these cases a number of the plants had died altogether of fungoid disease. In no case, however, was the average increase in any of the field soil series less than  $5\frac{1}{2}$  per cent.

The next lowest average increase obtained in any of the 44 series was 77 per cent., while an average increase of as much as 603 per cent. was obtained from one series of boxes of garden soil after steaming, and 403 per cent. from one of the field soil series, the average over the entire set of 44 experiments being 235 per cent. Fig. 2 shows the total crops obtained from all the series both from steamed and from untreated soil.

No noticeable effect can be traced which would indicate that enemies to plant growth capable of being destroyed by partial sterilisation, congregate at a certain depth of the soil, or move up and down according to the season of the year. There is some indication that where the soil is

capable of yielding a fair crop, the percentage of increase obtained by steaming will be greater than where the soil is very deficient in plant food. The richer garden soil when steamed, gave on the whole, as will be seen from the diagrams, a greater increase than the field soil, while in the latter, little reaction was ever obtained below 12 inches, where soil not previously disturbed was reached.

The level at which the maximum increase was obtained varied considerably in each experiment, being by no means confined to the layers near the surface. Fig. 3 shows that while in the garden soil the maximum

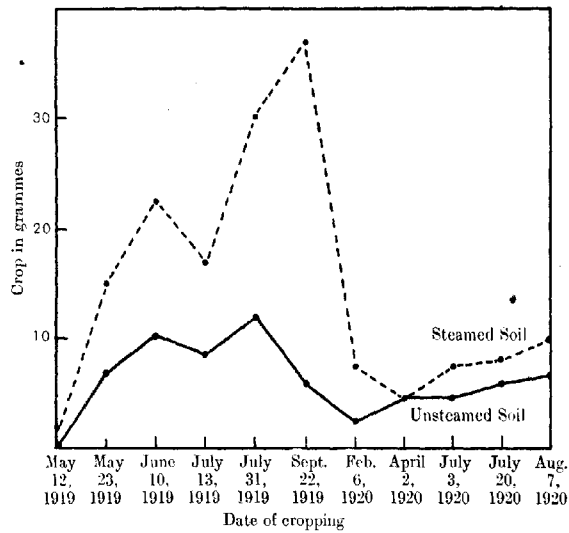


Fig. 5. Curves showing total crop obtained from steamed and unsteamed soil. Crops grown in large boxes.

increase obtained varied from the surface layer to that 16 or 18 inches below the surface, in the field soil the maximum was always obtained in the top 10 inches, and rarely below 8 inches, except in one case (the 15th experiment) where the whole crop from both treated and untreated soil was much retarded and very poor.

It would further appear that the benefit obtained by steaming is not confined to the first crop sown, as will be seen from Fig. 4, which illustrates the results obtained from sowing five successive crops on the same soil.

These boxes and the controls were sown again as soon as the first crop had been harvested, and then, after the second crop had been cut, the soil was steamed a second time and re-sown. All three sowings gave an increase of crop from the steamed soil, but a fourth crop which was sown without further steaming showed no advantages over the control. After

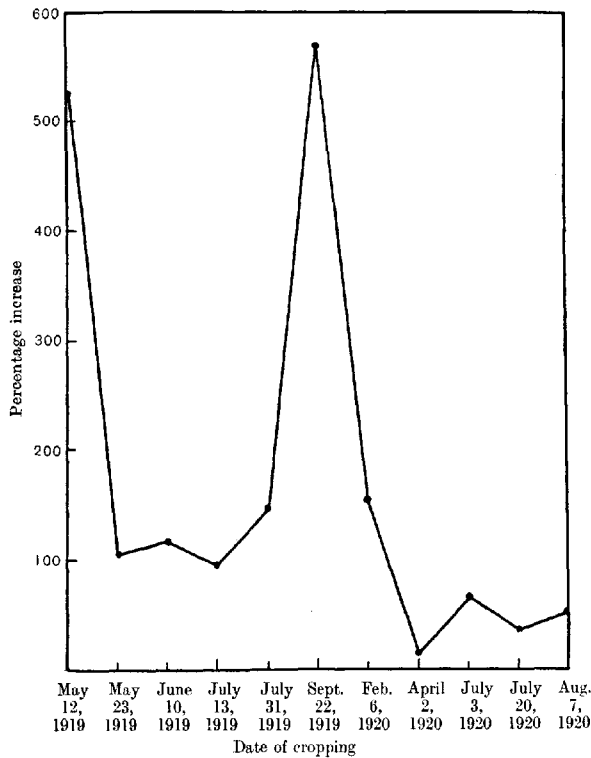


Fig. 6. Curve showing percentage of crop increase on steamed over unsteamed soil.  
Crops grown in large boxes.

this had been harvested, the soil was steamed a third time and sown again. The fifth crop showed some increase over the control, but these boxes were largely a failure, owing to disease, as is shown by the dotted line.

During 1919-1920 a second set of experiments was carried out in larger boxes, each containing a cubic foot of soil. The soil used was from

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the field previously mentioned, and the results are shown in Figs. 5 and 6—Fig. 5 giving the actual crops obtained in each of 19 experiments, from treated and untreated boxes, and Fig. 6 the percentage of increase in each case. The increases are generally speaking smaller than in the shallow boxes, and during the exceptionally wet season of 1920 crops from both treated and untreated soil were much poorer than in the previous season.

Since the steaming of the soil on a practical scale, other than under glass, presents so many almost insuperable difficulties, experiments were also made in the treatment of soil by dry heat. Satisfactory results were obtained with soil which had been passed through a naked flame and raised approximately to between  $80^{\circ}$  and  $90^{\circ}$  C.

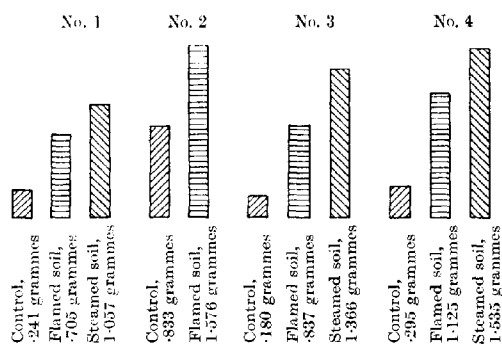


Fig. 7. Diagram showing crops obtained from soil flamed to a temperature of  $80^{\circ}$ – $100^{\circ}$  C., from untreated soil and from steamed soil.

The method adopted was as follows:

A fine stream of earth was passed through an inclined metal drum at one end of which was a flame from a blow lamp. The soil trickled through the flame, and the temperature was recorded as it fell out of the drum. Small scale experiments were first tried, using an apparatus made of a cylindrical biscuit tin and a plumber's blow lamp. For the large scale experiments out of doors, an apparatus was constructed capable of dealing with about a ton of soil an hour. Of this, the drum, which was about 18 inches in diameter, revolved, causing the soil in it to turn over and thus become thoroughly heated by flames from two large paraffin oil burners, blown by a fan.

The results from the soil thus heated were, on the whole, less good than in the case of the steamed soil, the temperature was lower, and the

soil less uniformly heated. Boxes of it were sown with mustard seed, together with controls of untreated soil, and of soil which had been steamed. The results are shown in Fig. 7.

Soil heated by electricity also gave good results provided a sufficient temperature was reached. Crops from soil raised to  $100^{\circ}\text{C}$ . by applying an alternating current to the boxes compared very favourably with those grown on steamed soil, and that to which dried blood had been added, both in conjunction with the steaming and without; the purpose of adding dried blood being to provide the soil, for comparison, with a manure rich in available nitrogen. The results are shown in Fig. 8.

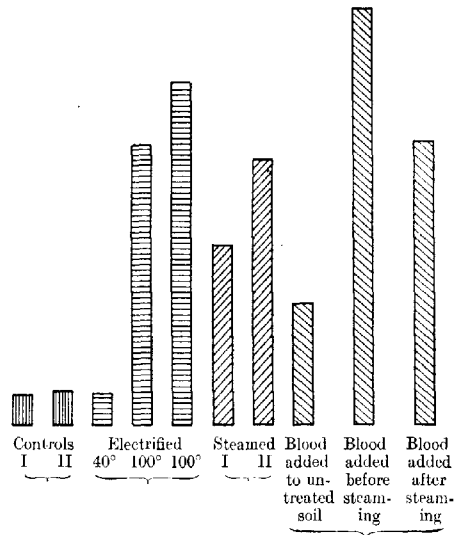


Fig. 8. Diagram showing crops obtained from soil heated by electricity to  $100^{\circ}\text{C}$ . Crops obtained from steamed soil with and without the addition of blood are also shown for comparison.

From the Figs. illustrating the results from steamed soil taken at varying depths, it appears probable that the maximum increase of crop obtained by steaming the soil occurs, in previously manured soil, at those depths where the manuring has been most thorough, and that the effect of partial sterilisation is to liberate some plant food, present but not otherwise available.

Dr Russell<sup>1</sup>, finding that partial sterilisation caused first a fall and

<sup>1</sup> *Soil Conditions and Plant Growth*, pp. 168-75.

# *Partial Sterilisation of the Soil by Heat*

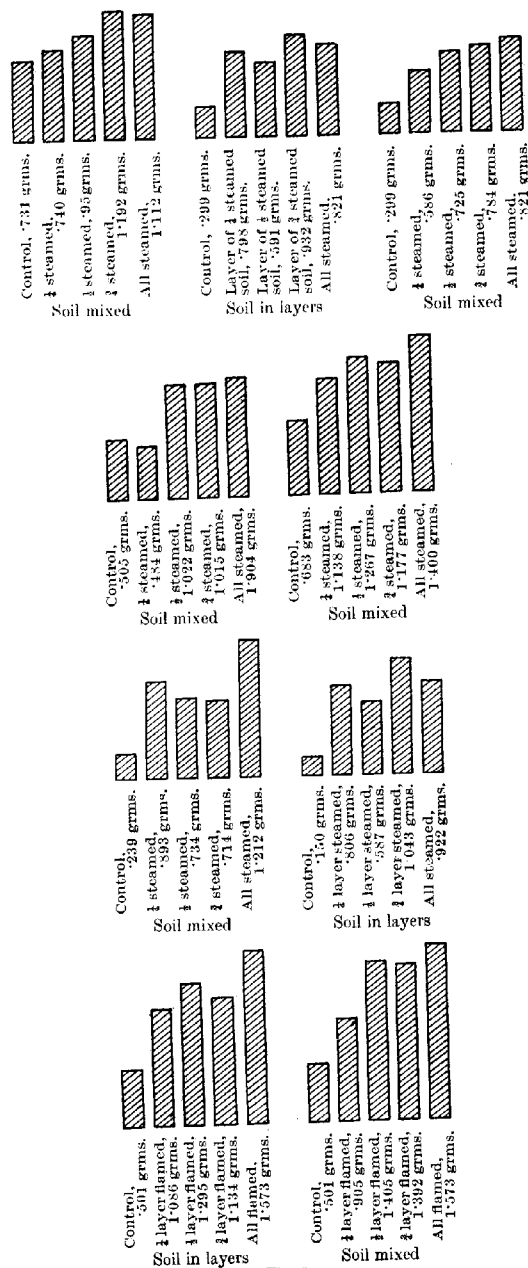


Fig. 9.

then a rise in the bacterial content of the soil, reached the conclusion that some factor, limiting the growth of bacteria favourable to plant life, was eliminated by partial sterilisation, and believed this to be the non-spore-bearing protozoa in the soil, amoebae and ciliates. He concluded that these when killed by heat or poisons leave the soil in a condition very favourable to the growth of other spore-bearing bacteria which are beneficial to plant life, and which rise in numbers after the destruction of their enemies, their spores being capable, unlike the protozoa, of resisting the temperature of steam.

If the benefits of partial sterilisation by steam consisted solely in the destruction of protozoa, the reintroduction of a small quantity of untreated soil into the heated mass should counteract the effects of steaming. In order to ascertain if there were not some other factor at work, the following experiments were carried out. Crops of mustard were sown in boxes of a mixture of treated and untreated soil. The soil having been steamed or flamed in the manner previously described, a layer was placed in boxes, the soil below being untreated. The layer varied in thickness from one-quarter of the total amount of the soil in the box to three-quarters. Boxes were also set up with similar proportions of treated and untreated soil thoroughly mixed together. In each experiment a box of untreated soil and one of soil altogether steamed or flamed was sown.

The addition of untreated soil did not mask the effect of the heat treatment. As will be seen in Fig. 9, which illustrates seven such experiments made, the increase of growth varied, generally speaking, in proportion to the amount of soil in the box which had been subjected to treatment. So far from the reintroduction of untreated soil having any effect, the crops in some cases exceeded those from soil wholly treated. It is clear therefore that the decomposition of soil materials effected by the heat is a potent factor. A further possible explanation may lie in some unascertained fact, such as that the protozoa, and possibly other living organisms, when dead become, instead of enemies to the spore-bearing bacteria in the soil, a food for them and for the plant.



**APPENDIX. Individual Weights (in grammes) of 22 Crops grown on soil at different levels with percentages of increase.**  
*Garden Soil.*

Depth in In- Exp.	0-2			2-4			4-6			6-8			8-10			10-12			12-14			14-16			16-18		
	Con- trol	In- crease	%	Con- trol	In- crease	%	Con- trol	In- crease	%	Con- trol	In- crease	%	Con- trol	In- crease	%	Con- trol	In- crease	%	Con- trol	In- crease	%	Con- trol	In- crease	%	Con- trol	In- crease	%
1	1784	2431	19	1194	1754	47	583	1295	55	357	3993	617	1372	2410	54	547	1467	168	2355	1332	0	352	889	117	334	784	131
2	330	567	72	104	976	463	283	1773	542	265	1238	504	247	818	231	176	1247	68	258	457	151	352	889	117	334	784	131
3	482	1734	260	316	216	105	317	2971	837	345	1466	325	474	1884	287	103	1063	492	490	1407	177	335	483	124	348	724	113
4	536	2984	471	331	1559	371	323	1911	494	558	1582	342	269	2037	267	106	1254	655	493	1162	221	336	483	124	348	724	113
5	466	1258	171	238	1059	351	323	1911	494	558	1582	342	269	2037	267	106	1254	655	493	1162	221	336	483	124	348	724	113
6	479	1258	171	238	1059	351	323	1911	494	558	1582	342	269	2037	267	106	1254	655	493	1162	221	336	483	124	348	724	113
7	437	1315	201	221	1233	680	274	1449	429	256	1738	638	211	1477	562	137	1272	708	517	1217	544	194	878	553	122	752	111
8	512	1720	236	205	1200	485	332	2618	688	109	1721	1179	304	1039	388	347	1498	988	284	1337	555	142	852	300	227	1533	716
9	372	2028	449	720	1474	349	171	708	307	223	1224	492	258	1205	392	121	849	982	204	1337	555	142	852	300	227	1533	716
10	606	1270	109	326	836	15	489	1852	275	45	379	504	141	1399	211	137	1029	25	463	293	171	505	1500	107	336	518	54
11	391	1461	273	250	1158	363	267	1031	393	310	1753	449	1486	1716	53	1041	1240	650	204	1449	290	551	1401	11	462	2520	117
12	656	1271	93	400	1411	32	413	1657	48	208	1065	921	149	1511	842	103	1033	133	166	1467	872	55	1401	11	462	2520	117
13	574	1798	213	208	1485	344	175	1659	842	298	1405	671	151	1717	421	249	1025	673	166	1467	872	55	1401	11	462	2520	117
14	798	2735	242	208	1485	344	175	1659	842	298	1405	671	151	1717	421	249	1025	673	166	1467	872	55	1401	11	462	2520	117
15	504	2968	598	210	1325	961	208	1737	482	387	1407	315	117	1366	227	574	1358	375	303	1266	14	335	463	124	348	724	113
16	706	2914	289	423	1341	275	733	1449	97	573	2408	329	101	2405	222	552	1495	113	389	1400	283	384	1752	356	350	1097	178
17	604	1520	151	416	1586	281	388	1658	327	416	1649	296	123	1403	418	428	1493	113	389	1400	283	384	1752	356	350	1097	178
18	419	2144	411	505	1693	235	845	1469	77	1870	3408	55	123	1403	418	428	1493	113	389	1400	283	384	1752	356	350	1097	178
19	387	1738	349	422	1312	210	355	1395	250	511	1700	151	128	1403	418	428	1493	113	389	1400	283	384	1752	356	350	1097	178
20	747	1556	108	418	1717	283	426	1395	250	511	1700	151	128	1403	418	428	1493	113	389	1400	283	384	1752	356	350	1097	178
21	698	1525	118	806	2043	153	930	2068	115	420	2045	155	606	1764	153	403	1700	191	254	1857	729	358	1534	328	526	1755	227
22																											

*Field Soil.*

1	247	1063	390	283	1421	402	439	1867	325	301	1493	293	254	673	165	207	769	243	298	573	175	224	417	86	221	434	96
2	520	2062	514	225	1850	722	190	1445	600	187	2039	390	302	1314	443	153	453	516	108	170	496	125	429	218	149	380	261
3	136	446	228	206	747	1032	385	289	16	184	1715	288	265	900	361	126	1256	455	108	134	456	131	452	131	456	131	97
4	408	189	166	212	540	123	608	710	16	184	1715	288	265	900	361	126	1256	455	108	134	456	131	452	131	456	131	97
5	408	189	166	212	540	123	608	710	16	184	1715	288	265	900	361	126	1256	455	108	134	456	131	452	131	456	131	97
6	479	1278	166	508	1613	217	416	1429	243	170	1925	345	319	1434	479	152	1626	384	190	281	735	161	197	572	190	202	816
7	484	1273	167	1182	1218	3	623	1179	89	562	2238	345	319	1434	479	152	1626	384	190	281	735	161	197	572	190	202	816
8	454	2123	367	1333	2860	162	877	2240	536	362	2238	345	319	1434	479	152	1626	384	190	281	735	161	197	572	190	202	816
9	407	1476	143	201	1672	474	386	1491	283	435	1387	226	307	812	164	296	733	298	305	399	30	216	517	190	207	816	
10	798	2054	178	305	1825	276	534	2048	452	450	1848	304	357	1251	250	388	1558	301	471	746	178	422	397	192	541	1020	201
11	385	2107	447	443	1552	257	382	1977	417	450	1848	304	357	1251	250	388	1558	301	471	746	178	422	397	192	541	1020	201
12	211	5716	269	417	2209	480	508	1553	203	409	1475	290	403	1409	144	274	809	267	491	761	178	422	397	192	541	1020	201
13	639	2783	281	483	1697	313	732	2050	180	474	1878	298	403	1409	144	274	809	267	491	761	178	422	397	192	541	1020	201
14	413	1668	163	491	1915	86	950	1165	21	373	1278	74	557	2952	75	378	983	162	392	424	65	131	355	155	337	619	80
15	455	1668	163	491	1915	86	950	1165	21	373	1278	74	557	2952	75	378	983	162	392	424	65	131	355	155	337	619	80
16	455	1668	163	491	1915	86	950	1165	21	373	1278	74	557	2952	75	378	983	162	392	424	65	131	355	155	337	619	80
17	432	837	91	384	1247	224	373	1111	191	549	2431	315	315	2431	315	315	2431	315	315	2431	315	315	2431	315	315	2431	315
18	432	837	91	384	1247	224	373	1111	191	549	2431	315	315	2431	315	315	2431	315	315	2431	315	315	2431	315	315	2431	315
19	484	1586	1355	349	1770	120	324	1686	219	427	1040	373	347	881	153	464	1083	406	253	351	223	423	372	2289	518	341	177
20	484	1586	1355	349	1770	120	324	1686	219	427	1040	373	347	881	153	464	1083	406	253	351	223	423	372	2289	518	341	177
21	557	2148	298	500	1719	612	675	2124	258	570	1758	190	472	1606	192	120	1245	288	1000	521	48	470	236	278	428	294	426
22	557	2148	298	500	1719	612	675	2124	258	570	1758	190	472	1606	192	120	1245	288	1000	521	48	470	236	278	428	294	426

(Received 24th January 1921.)

THE FACTORS DETERMINING SOIL  
TEMPERATURE.

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(With Thirteen Figures in Text.)

## INTRODUCTION.

THE general principles regulating soil temperature are well known.

Measurements in deep mines and wells indicate the existence of a temperature gradient in the earth's crust from the interior outwards, which causes a flow of heat to the surface at a sensibly constant rate. Extensive measurements were carried out by Forbes<sup>1</sup> and by William Thomson<sup>2</sup> (afterwards Lord Kelvin) who worked up the former's experimental values, and later by a Committee of the British Association, which concluded that an average of "41.4 gramme-degrees of heat escape annually through a sq. cm. of a horizontal section of the earth's substance<sup>3</sup>." For our present purpose this source of heat is negligible.

Superimposed on this are much larger temperature fluctuations due to radiation from the sun. During the day the sun's heat warms the surface of the earth, thus setting up a difference of temperature between it and the subsoil. This causes a heat wave to be propagated into the soil.

The difference between the minimum and succeeding maximum temperature at any given depth is defined as the amplitude of the temperature wave at that depth. The wave travels comparatively slowly, and its amplitude decreases rapidly with increasing depth of soil, and at a depth of about 3 ft. the daily fluctuations of temperature are inappreciable; in other words, the wave is considerably damped in its passage through the soil.

A heat wave is manifested by the rise of temperature that it causes, and this is determined by the specific heat of the various layers of soil: if the soil is moist the specific heat is increased and a smaller temperature rise takes place than in dry soil. Fluctuations at the surface of the soil take some hours to travel even to a depth of 6"; the minor ones are

<sup>1</sup> *Trans. Roy. Soc. (Edin.)*, **16** (1846), pt. 2, p. 189.

<sup>2</sup> *Ibid.* **23** (1862), p. 157.

<sup>3</sup> *Brit. Assoc. Rept.* (1882), p. 72

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completely damped out even in this short distance and become inappreciable to our instruments.

During the night the reverse process takes place: the surface loses heat by radiation, and there is a flow of heat from the interior outwards, but again the rate of travel is slow and there is much damping. The night phenomena are not the precise reverse, but differ from those of the day.

The surface of the soil experiences generally a daily rise and fall of temperature. The propagation of the temperature wave into the soil results in the temperature at any given depth exhibiting a fluctuation which is a kind of reduced image of that at the soil surface. The time period of this wave (i.e. the period of time between successive minima or maxima) remains practically unaltered, but the amplitude, or temperature range, is diminished and there is a "time-lag"—amounting to several hours at the 6" depth,—between the initiation of a temperature change at the surface and its appearance at a given depth of soil.

The purpose of this paper is to discuss the factors influencing soil temperature and the extent to which other measurements such as air temperature, hours of sunshine, etc., can be made to give information as to soil temperature in cases where direct determinations have not been made.

The material of this discussion is provided by readings (taken to 0.5° C.) of a continuous self-recording thermometer buried at 6" in the soil at Rothamsted, and by a series of other thermometers, exposed in a screen open to the air, or buried in the soil, on which readings are taken at stated intervals. These instruments, together with sunshine and radiation recorders which were also used, are described in Appendix I.

The soil above the thermometer was carefully kept free from all plant growth. The period dealt with is from Dec. 22nd, 1913, to Dec. 23rd, 1914. The examination of the data was suspended during the War, although the records have been, and will be, continued.

The season was characterised by a dry January, a very wet February and March—7.55" of rain falling in these two months as against an average of 3.37"; a dry sunny April with cold N.E. winds; a dry cold May with N.E. and N.W. winds and several frosts; a warm June, the prelude to a warm, dry, sunny summer, there being 1211 hours of sunshine over the season April to September inclusive; a wetter October; a cold November and an extraordinarily wet December.

## THE WARMING OF THE SOIL.

Inspection of the curves shows two distinct kinds of curves—one obtained in summer and the other in winter.

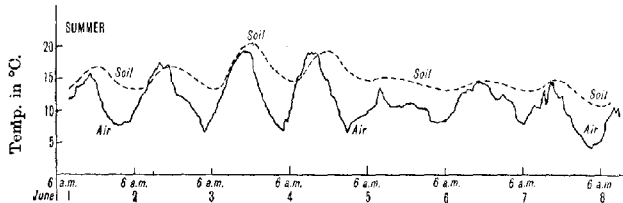


Fig. 1 (a).

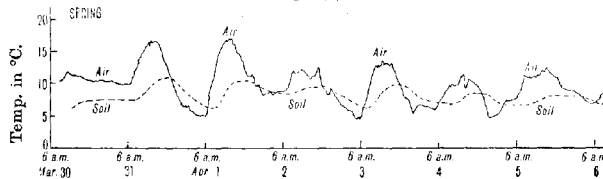


Fig. 1 (b).

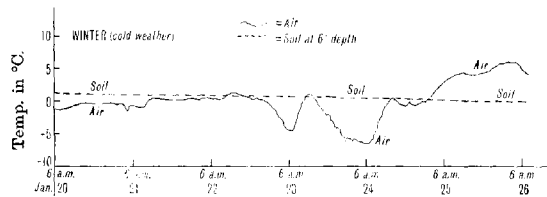


Fig. 1 (c).

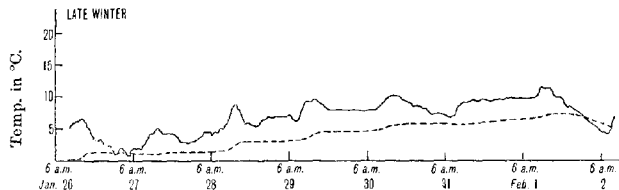


Fig. 1 (d).

*Summer records.* Typical records for the spring and summer months are shown in Fig. 1b and 1a. From these it appears that the rise of temperature which begins at the surface soon after daybreak does not penetrate to a depth of 6" until about 9 a.m. (Greenwich mean time)<sup>1</sup>. The soil

<sup>1</sup> All times throughout this paper are Greenwich mean time.

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temperature then rises fairly rapidly till about 4.30 p.m., remains steady, or rises only slightly, for about an hour, and then from about 5.30 p.m. falls slowly and continuously throughout the next 16 hours till about 9 a.m. the next day. In spring and autumn, however, there is a period during which the maximum temperature occurs on the average about 4.30 p.m. (Fig. 2).

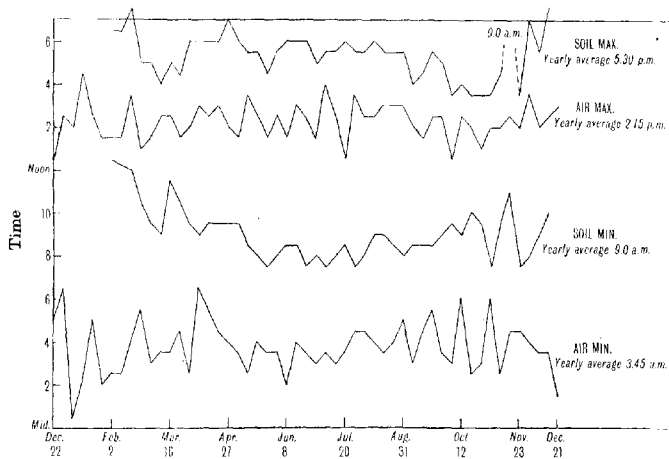


Fig. 2. Weekly averages of daily times of min. and max. temperatures for air and soil.

The sun of course reaches its meridian at 12 noon; the air temperature reaches a maximum about 2.15 p.m., and then falls. The steady period in the soil from about 4.30 p.m. to 5.30 p.m. indicates that accretions of temperature are still continuing, but they are now balanced by losses, so that on the whole there is neither gain nor loss. Another steady period often occurs at the time of the minimum, indicating that the cooling process is now being counterbalanced by additions of temperature.

We have subjected these steady periods to a careful examination, but have found no simple relationship between their times of duration and the weather conditions.

At a depth of about 6" the warm period of the day is, broadly speaking, between noon and midnight, while the cool period is from nearly midnight till just before noon. In so far as temperature is a determining factor the activity of soil microorganisms might be expected to be greatest in the afternoon and evening and least in the morning.

The warming of the soil is much more rapid than the cooling: it takes only about seven hours to attain the maximum, but the effect persists for another hour. This phenomenon was observed also by Callendar<sup>1</sup> at the McGill University, Montreal. Between his results and ours, however, there is an interesting difference in detail; in our case the rise of temperature at 6" depth is rapid and the fall slow; in his the rise at 10" depth is slower than the fall, and the crest of the wave exhibits in some cases signs of breaking into two.

For a considerable part of the summer the maximum soil temperature at 6" depth fluctuated round about 22° C.: the daily values rose as high as 26.5° C., and the weekly averages reached 24° C. (Fig. 3).

*Winter records.* The records for the winter differ completely from those obtained in the summer. The soil temperature in December and January normally shows very little daily variation, sometimes when the surface soil is frozen the record is a straight line for days together (Fig. 1c); such fluctuations as appear are spread over several days. Towards the end of January daily variations begin to show; there is a perceptible daily rise, but frequently no fall, so that the charts appear to be a succession of upward steps (Fig. 1d). In March the daily fluctuations invariably occur, though there is still a tendency for a several day period to appear concurrently. During the winter months the maximum temperature fluctuated around 6° C.

The factors determining the amount and the nature of the temperature rise are the sunshine, the soil moisture and the prevalence of cold winds.

*Effect of sunshine.* Two methods are adopted at Rothamsted for measuring the sunshine: the Wilson radiointegrator, in which the solar radiation is estimated by the amount of evaporation of blackened alcohol contained in a bulb exposed to the open sky, and the Campbell-Stokes sunshine recorder, giving the number of hours of bright sunshine in each day (see Appendix I).

An examination was made of the daily records of sunshine and soil maximum temperature to see what relationship existed between them. In general it was found that they increased and decreased together, but there were many exceptions; the number of these is greater in winter than in summer. The actual numbers of exceptions in each of the periods into which the records were divided for convenience in examination is as follows:

<sup>1</sup> *Trans. Roy. Soc. Canada*, 1895 (2nd Series), 1, p. 1.

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20-day periods		35-day periods								10-day period
Feb. 2	Feb. 18	Mar. 9	Apr. 13	May 18	June 22	July 27	Aug. 31	Oct. 5	Nov. 9	Dec. 14
to	to	to	to	to	to	to	to	to	to	to
Feb. 18	Mar. 9	Apr. 13	May 18	June 22	July 27	Aug. 31	Oct. 5	Nov. 9	Dec. 14	Dec. 23
All dates	3	5	11	10	7	10	14	14	14	4

From September onwards, during the winter months, the exceptions occurred on 40 per cent. of the days, and during early February the relationship was completely inverted. The exceptions are, of course, of two types: the maximum soil temperature may decrease although the hours of sunshine increase, or it may rise when the hours of sunshine fall. Expressed in statistical terms, the correlation coefficient for the normal relationship between maximum temperature and hours of sunshine for the whole period (Feb. Dec.) works out to  $.434 \pm .032$ , which is not particularly high. Strictly speaking account should be taken only of the hours of sunshine up to noon, as sunshine occurring after that time cannot directly affect the soil maximum temperature on the same day. When this correction is made the correlation coefficient is  $.357$  which is lower than the value for the uncorrected figures. The reason for this, assuming that the difference is significant, is not at all clear and needs further investigation. It is possible that part of the heat is carried into the soil by the downward percolating water, as well as by thermal conduction from soil particle to soil particle. Theoretical considerations indicate, and Bouyoucos<sup>1</sup> has shown experimentally, that the temperature changes in the top layers of soil would directly influence the rate of percolation because this depends on the viscosity and surface tension of the soil solution, both of which are decreased by increase in temperature. For instance, if the rate of percolation became greater than the rate of travel of the true temperature wave it would be possible for a change of temperature in the top layers of soil, occurring after midday, to contribute to the maximum temperature recorded by the 6" soil thermometer.

*Effect of rainfall.* It is difficult to estimate this accurately because rain necessarily comes at a time when the sun is not shining, and therefore when the temperature of the soil is likely in any case to be adversely affected.

From early spring to the end of the summer, however, rain cools the soil or stops a warming that would otherwise be expected to occur. We have carefully examined the records for any indication of a warming

<sup>1</sup> Michigan Expt. Station, *Technical Bull.* No. 22 (1915).

effect said to be produced in spring, but we find evidence of such action only once (March 8th).

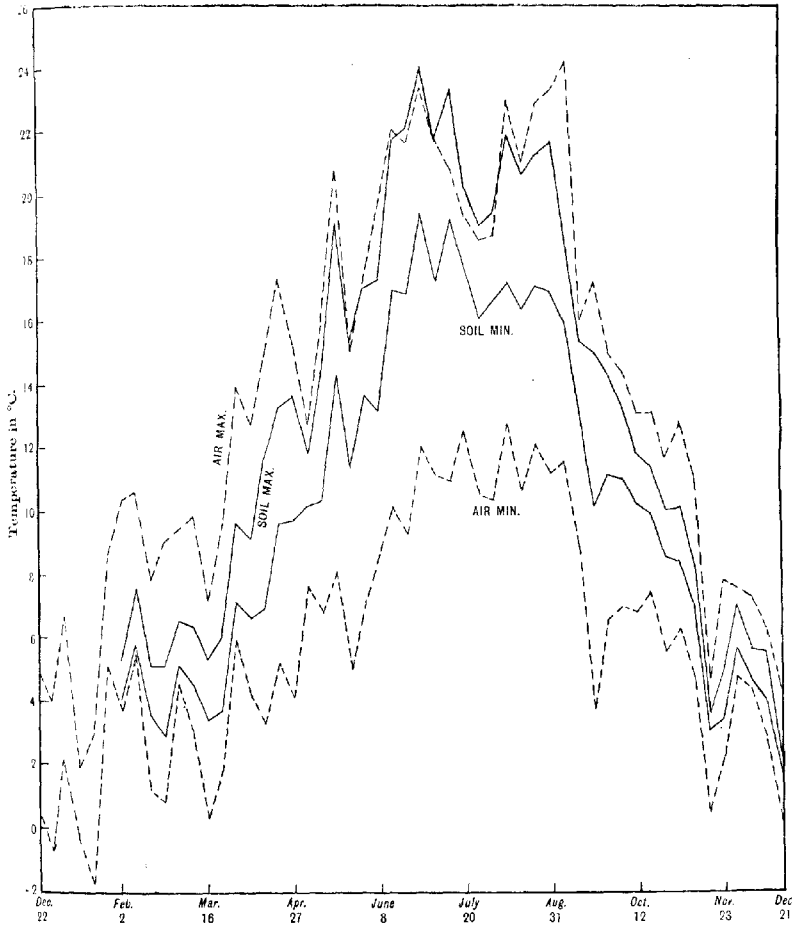


Fig. 3. Weekly averages of daily min. and max. temperatures in air and soil.  
(Continuous lines=soil temperatures; broken lines=air temperatures.)

*Effect of wind.* There is no definite evidence that drying winds reduce the temperature of the soil at 6" depth. The rule seems to be that during



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periods of drought the soil temperature is closely related to sunshine; during periods of rain the soil temperature is lowered; in late autumn, however, it falls to a less extent than would otherwise have happened.

*The relationship between soil maximum and air maximum temperature.* It is well known that during hot sunshine the surface of the soil is considerably hotter than the air thermometer in the screen. At a depth of 6" however the temperature wave is so damped down that the maximum temperature in the soil is usually almost that of the air during the summer, being sometimes a little higher and sometimes a little lower. In winter, however, during the period December to March, it is 3° C. lower. The weekly averages are plotted in Fig. 3. Reference has already been made to the circumstance that the soil maximum is attained about three hours later than the air maximum (Fig. 2).

*The cooling of the soil.* The cooling of the soil takes place during the night, beginning at about 5.30 p.m. and continuing till some time between 7.30 a.m. and 10 a.m.—being thus much slower than the warming. The meteorological data are insufficient to allow detailed discussion of the effect of various conditions—no observations being taken at Rothamsted during the night.

The fall is greater on clear nights than on cloudy nights. In late autumn rain slows down the rate of fall. During October and November for example the temperature is falling steeply, but on wet days there is a distinct slackening in the rate of fall; e.g. on November 2nd rain stopped the fall altogether, and once—on November 30th—rain seemed even to warm the soil.

In view of the relation between sunshine and soil temperature it might be expected that long hours of sunshine on one day would be associated with a high minimum soil temperature on the next. This, however, happens no more often than in 50 per cent. of the cases in spring and summer while in early winter the reverse happens, and a sunny day is followed by a clear night during which there is considerable cooling of the soil by radiation.

The cooling of the soil, unlike the warming, never proceeds as far as that of the air in the screen, the difference is usually considerable, not infrequently of the order of 6°–8° C.; it is greater in summer than in winter. While the air minimum in the screen is not infrequently below 0° C. in winter and has a weekly average fluctuation round about 2° C., the soil at the depth of 6" rarely attained 0° C., and the weekly average of its minimum temperature was about 4° C.

There is a further difference between the cooling of the air in the

screen and that of the soil. In the case of the air the coldest point is reached at approximately the same time in summer as in winter, viz. at 3.45 a.m.: this would of course hardly be expected, but the records show that it is so. In the case of the soil at 6" depth the lowest temperature is reached much earlier in summer than in winter—on an average at 7.45 a.m. as against 10.30 a.m. in winter, with intermediate values in spring and autumn; the soil in fact indicates more faithfully than the air the earlier rising of the sun in summer (Fig. 2). In summer the minimum temperature was usually about  $18^{\circ}\text{C}$ ., rising, however, on one day, to  $21^{\circ}\text{C}$ .; in winter it is usually about  $4^{\circ}\text{C}$ .

*The mean temperature of the soil.* The mean temperature of the soil at the 6" depth is immediately deducible from the maximum and minimum temperatures; it was usually about  $20^{\circ}\text{C}$ . during the summer months and about  $5^{\circ}\text{C}$ . in winter. Since it involves both maximum and minimum temperatures, i.e. both heating and cooling processes, it is more complex than either and a less clear connection exists with sunshine, rainfall and other conditions.

The radiometer readings are more closely associated with the mean soil temperature than are other readings we have taken. At low minimum periods there is disagreement as might be expected, but when these are eliminated a fairly good relationship can be seen. The connection is traceable even in February when the sunshine shows little relationship to soil temperature; it is only slight during March, April and early May (during which period sunshine and radiometer agree fairly closely with each other). It is more marked in later May and June; sunshine and radiometer now begin to show some disagreement, but the soil mean agrees better with the radiometer than with the sunshine.

The relationship between radiometer readings and soil mean temperature is, however, qualitative only. Both quantities on the whole tend to rise or fall together, but not to the same extent; we were unable to deduce the daily fluctuations in value of mean soil temperature from the radiometer readings.

The effect of sunshine is less than one would expect; hours of sunshine and maximum soil temperature frequently vary in the same direction, but the quantitative relationship is less marked, the correlation coefficient being  $+0.434$  (p. 216). The number of hours of sunshine has considerably less effect on the minimum, and therefore on the mean temperature of the soil. On about 50 per cent. of the occasions there is no actual relationship between hours of sunshine and mean temperature, although of course it is always true that sunshine warms the soil. During winter,

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on the other hand, there are periods where long hours of sunshine are accompanied by no rise, but an actual fall, in the mean temperature of the soil, the reason being the intense cooling by radiation during the clear nights that usually accompany sunny days. The number of days where hours of sunshine apparently show no relationship with the mean soil temperature is as follows:

	Feb. 2 to Feb. 18	Feb. 19 to Mar. 8	Mar. 9 to Apr. 12	Apr. 13 to May 17	May 18 to June 21	June 22 to July 26	July 27 to Aug. 30	Aug. 31 to Oct. 4	Oct. 5 to Nov. 8	Nov. 9 to Dec. 13	Dec. 14 to Dec. 23
No. of days	17	18	35	35	35	35	35	35	35	35	10
No. when no apparent relationship	All but one	5	12+3	16+2	13+5	12+3	18+2	21+1	22+4	10+4	4+1

The larger figures in the lower line give the number of days when an increase in number of hours of sunshine was accompanied by a fall in the mean soil temperature, or vice versa. The small added figures refer to cases where the number of hours of sunshine changed from one day to another, but without change in mean temperature of the soil.

We may conclude that in the first six months of the year the number of hours of sunshine largely determines the mean soil temperature, but in the latter part of the year the hours of sunshine have less effect; cooling by radiation at night now becomes prominent.

*Effect of moisture.* Rain, as already shown, reduces the maximum temperature, but in autumn it also tends to raise the minimum; the effect on the mean temperature is therefore somewhat less than might be expected. The direct relationship should be sought in the percentage of soil moisture rather than in the amount of rainfall; but it was not possible to determine the moisture at regular intervals in the soil overlying the thermometer; to have attempted this would have affected the temperature readings. In another connection, however, frequent soil moisture determinations were made during the period under review on two of the fallow plots in Broadbalk field. The data have already been published<sup>1</sup> and need not be repeated here. As the plots do not adjoin the soil thermometer no detailed comparison is possible. The data show, qualitatively, that the moisture variations are inversely related to the mean temperature; the soil warms as it dries, and cools as it becomes moist.

*The warming of the soil in spring.* The curves show that the soil temperature rises much more rapidly during the spring than might have been expected from consideration of sunshine alone. The rapid rise however does not occur until the soil has become drier. Apparently therefore

<sup>1</sup> E. J. Russell and A. Appleyard, this *Journal*, 1915, 7, p. 1

the result is due to the combined action of two causes each reinforcing the other—the increase in the sunshine coinciding with a decrease in soil moisture.

*The cooling of the soil in early winter.* This also proceeds very rapidly and it is not hastened but retarded by the wetting of the soil. Cooling is probably due to the great amount of radiation taking place during the fine October nights.

#### RELATIONS OF SOIL TEMPERATURE AND BIOCHEMICAL ACTIVITY.

Our results show that the soil organisms are living under distinctly warmer conditions than would be inferred from the air temperature readings, and under much more equable conditions than the plants or animals on the surface of the soil; they are not liable to the same amount of cooling while they enjoy as high or higher maximum temperatures. During the summer months the moisture and temperature conditions somewhat resemble those of an incubator kept at 20° C.; the actual temperature range is from 24° C. to a rare minimum of 16° C., the corresponding air figures being 24° and 9° C. with a mean value of 16° C. In winter the temperature is low, but it is well above that of the air and only rarely was the freezing point attained.

The great increase in production of carbon dioxide sets in when the soil temperature exceeds 5° or 6° C.; after that the two quantities increase together. Increase in nitrate production appears to begin a little later when the temperature has reached 7° to 9° C.

Unfortunately the biochemical observations are insufficient to allow us to say with certainty whether high temperature amplitudes affect biochemical changes.

#### SUBSTITUTES FOR SOIL TEMPERATURE READINGS.

It would often be of advantage to soil investigators, ecologists and others to have some information about the soil temperature, but in few cases is this obtainable, self-recording soil thermometers being very unusual instruments. We have examined two possible substitutes—the 12" soil thermometer, and the air temperature readings.

*The relation of the present readings to those taken on the 12" "lagged" soil thermometer.* A 12" soil thermometer of the type ordinarily sent out by the Meteorological Office is embedded in the ground a few feet away from the 6" recording thermometer and readings are taken daily at 9 a.m. The figures do not reflect the mean value at the 6 inches; they more faithfully represent the minimum value at that depth.

It seems probable that the fairly close agreement between the 9 a.m. reading of the 12" soil thermometer and the minimum temperature at the 6" depth—which we have seen usually occurs at about the same time (9 a.m.)—is due to the effect of the thick iron tube in which the former is suspended. This tube is a good conductor of heat, hence the temperature inside it is at any time approximately the average temperature of the whole of the 12" depth of soil through which it passes. This average temperature is probably not greatly different from the actual temperature of the mid depth (6") at the same time. Hence, if the 12" thermometer is read at 9 a.m. we should expect a value approximately equal to the minimum reading at the 6" depth.

There are however several points in favour of the 12" thermometer—its cheapness, the ease with which it can be read, the fact that long series of readings already exist. We think these readings serve a sufficiently useful purpose to justify continuation, but the student should consider the results as a record of the 6" depth minimum soil temperature and not of the mean value.

*Relation of soil temperature to air temperature.* Since the air temperature is determined by the temperature of the soil surface it should show some relationships with the soil temperature at 6" depth.

Relationships are seen both in time changes and in temperature changes. Fig. 2 shows the weekly averages of the daily times of maximum and minimum temperatures both for air and soil. The two curves referring to the air readings show very little variation throughout the year, the mean curve through the points being in each case a straight line parallel to the date-axis. That this should be so for the air maximum is not surprising as the air takes up the appropriate temperature with very little lag. But the air minimum values would be expected to reflect, to a greater extent than the curve shows, the earlier rising of the sun in the summer months; the smooth curve through the points should, in fact, be more concave upwards than it actually is. The portion April 6th to Sept. 2nd does show this tendency, but not to any marked extent. The soil maximum curve is similar to the air maximum, in that it fluctuates very little about a mean value of 5.30 p.m., although towards the end the variations are somewhat larger. The soil minimum, on the other hand, is concave upwards over practically the whole range, and thus reflects better than the air minimum the earlier rise of the sun in summer. This is not surprising, as comparatively small temperature effects, due to early clouds, wind, etc., which have considerable influence on the air minimum, are largely damped out by the passage through 6" of soil.

It appears from Table I in Appendix II and from Fig. 3 that the *maximum* soil temperature, while much higher at the surface than the air temperature, is at a depth of 6" approximately the same as the maximum air temperature from June to August inclusive, though for the rest of the year it is somewhat below: the *minimum* soil temperature is some 6° to 8° C. above, the greatest difference being in the summer; and therefore the *mean* soil temperature is above the mean air temperature also. In our case the difference at 6" is about 3° to 5° C. in the summer; in winter there is little difference.

It has sometimes been stated that the mean soil temperature is approximately the same as the mean air temperature in the screen. This, however, is not correct. The statement is more nearly true of the readings taken at a depth of 12" at 9 a.m., using a lagged thermometer, and to this extent the soil minimum is approximately equal to the air temperature.

A fairly good estimate of the *maximum* temperature at 6" is obtained from the maximum air thermometer in the screen.

The soil 12" thermometer reading at 9 a.m. affords a fairly safe estimate of the minimum soil temperature. From these two quantities an estimate of the mean soil temperature can be obtained.

Where there is no soil thermometer it is impossible to obtain a reliable estimate. A rough approximation can however be made by assuming that the minimum soil temperature is about equal to the mean air temperature; or that the mean soil temperature is about 3° C. higher than the mean air temperature, this figure being too low at times of long sunshine and too high in times of rain.

#### THE PHYSICAL PROBLEM.

From the physical standpoint the problem is one of the propagation of a daily temperature wave into a porous material containing a variable quantity of water. The temperature at any point in the moist soil fluctuates, passing from a minimum value through a maximum, and back to a minimum in approximately 24 hours. Under these conditions we are concerned both with the conductivity of the material and its diffusivity. The conductivity is defined as numerically equal to the quantity of heat which flows in unit time through unit area of the material, of unit thickness, having a unit difference of temperature between its faces. The diffusivity (or thermometric conductivity) measures the change of temperature in unit volume of the substance which is

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produced by the same quantity of heat mentioned above in the definition of conductivity. It is thus numerically equal to  $\frac{\text{conductivity}}{\text{specific heat}}$ .

The diffusivity of the substance controls the rate at which a temperature wave is propagated in the material, and is thus very important in soil temperature problems since the temperature is almost always changing.

An analysis of temperature fluctuations at any point, caused by the daily temperature wave, would present no serious difficulty but for the complications introduced by the water around the soil particles. Patten<sup>1</sup> has investigated this problem. He finds that the conductivity of soil is increased by the addition of water, in spite of the low conductivity of the latter, owing to the better thermal contact between soil particles produced by the moisture film. For the same reason the diffusivity increases also. When the water content is still further increased, the temperature of the soil will rise more slowly in spite of the fact that heat may be conducted better than at a lower moisture content. This is due to the high specific heat of water, which is about five times that of dry soil. At a certain percentage of water content, therefore, we should expect a maximum value for the diffusivity. As the moisture content increases beyond the percentage it becomes the predominating factor, and the conductivity of the mixture gradually falls towards the value for water. Patten's investigations presented considerable experimental difficulties, and it is therefore worthy of notice that Callendar and McLeod<sup>2</sup> working on undisturbed soil under natural conditions have recorded an increase in diffusivity associated with an increase in moisture content.

In the following treatment we are concerned with the amplitude of the daily temperature variation. The amplitude varies considerably from day to day, but it is greater on clear than on cloudy days. Callendar makes the interesting suggestion that the amplitude at a depth of 4" might be taken as a measure of the mean clearness of the atmosphere or intensity of radiation for each day and night. Our observations show that there is a close relationship between both hours of sunshine and radiometer readings, and amplitude.

The correlation coefficient between hours of sunshine and amplitude is  $\cdot768 \pm \cdot0154$ ; that for radiometer readings and amplitude is  $\cdot877 \pm \cdot0087$ . High correlation therefore exists between these variables, especially in

<sup>1</sup> U.S. Bureau of Soils, *Bull.* 59 (1909).

<sup>2</sup> *Trans. Roy. Soc. Canada*, 1896 (2nd Series), p. 109.

the latter case. The radiometer gives the higher figure because its daily readings are the integration of the radiation throughout the 24 hours, whereas the hours of bright sunshine do not include cloudy periods, during which, especially in summer, considerable radiation may be reaching the earth's surface.

The weekly averages of the temperature amplitudes, both for air and soil, are shown in Table I in Appendix II, and Fig. 4. The continuous curves represent the amplitudes of rise, and the dotted ones the fall<sup>1</sup>. The curve shows the increase of both soil and air amplitudes associated with the summer months. An interesting feature is the close agreement of the amplitudes of fall and rise, in air as well as in

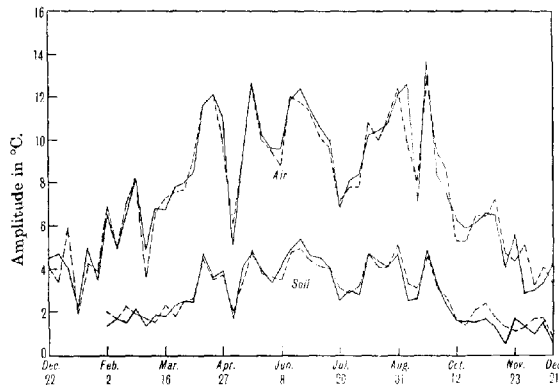


Fig. 4. Weekly averages of daily amplitudes for air and soil.  
(Continuous lines=amplitude of rise; dotted lines—amplitude of fall.)

soil. It would be incorrect, of course, to conclude that the average amplitude of fall for any week is equal to the amplitude of temperature rise for the same period, since this implies that the minimum weekly temperature remains constant throughout the year, which is obviously not the case. The agreement is due to the fact that the mean temperature rises slowly to its summer maximum, and falls slowly towards its winter minimum, so that on a weekly average the differences are inappreciable to our instruments.

The ratios of amplitudes of temperature rise and fall in the soil to those in the air have been examined in some detail: the data are set

<sup>1</sup> Amplitude of rise: maximum temperature of one day, minus minimum temperature of *same* day. Amplitude of fall: maximum temperature of one day, minus minimum temperature of *next* day.



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out in Table II in Appendix II, grouped in ratio steps of 0.5; they are also plotted in Fig. 5 which approximates to the normal type of frequency curve, showing that the ratio tends to have a certain mean value. The majority of the observations of both rise and fall come between .25 and .40, indicating that the soil amplitude is most likely to be between  $\frac{1}{4}$  and  $\frac{2}{5}$  of the air amplitude. The peak of the cooling curve is somewhat more to the right than that of the heating curve, which, if significant, indicates that for a given soil amplitude of rise or fall the corresponding

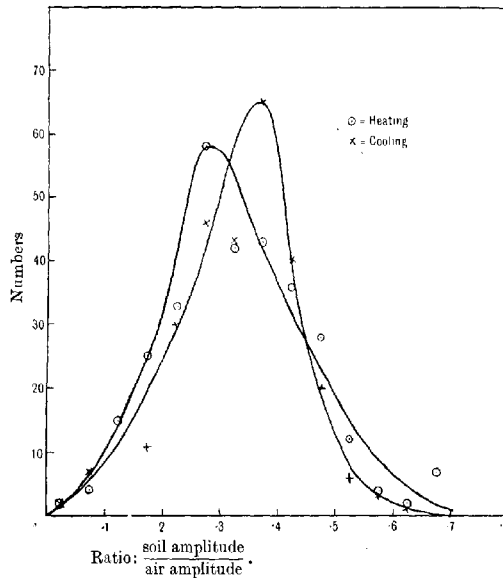


Fig. 5. Frequency curves for the ratio  $\frac{\text{soil amplitude}}{\text{air amplitude}}$ .

rise of air temperature is greater than the fall—a relationship which is explicable on physical grounds since the rise of the temperature of the thermometer in the screen is determined by upward convection currents and is therefore rapid, while the fall is determined by the slow process of diffusion with the lower layers of cool air.

The extent and causes of the deviation in value of the ratio of amplitude of soil and of air temperature can be studied from Figs. 6 and 7, giving the distribution of days, grouped by months, throughout the year which fall within a given range of amplitude. For the heating of the soil

the range .21 to .30 is found in nearly every month of the year, but most of the observations fall in two well-defined groups, the early summer and autumn. The ratios below this range are found most frequently in the winter months, while those above occur mainly in the summer. Hence the characteristic summer increase in the hours of sunshine and radiometer values will be associated with an increase in the ratio

$$\frac{\text{soil amplitude}}{\text{air amplitude}}$$

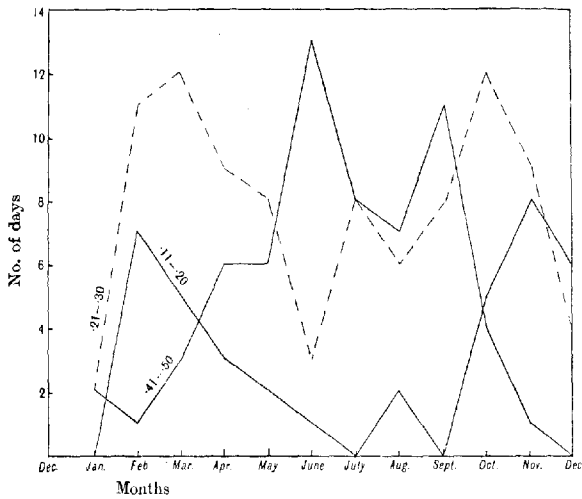


Fig. 6. Monthly distribution of given values of the ratio:  $\frac{\text{soil amplitude}}{\text{air amplitude}}$  [heating amplitudes].

The corresponding values for the ratio of the cooling amplitudes are distributed in a somewhat different manner; most of the days having a ratio above .31 to .40 (the ratio which included the peak of the cooling curve in Fig. 5) fall in the summer months, while the days on which the ratio is less than .31 are grouped in two well-defined periods, spring and autumn.

The average conditions for a given soil amplitude can be determined by studying the conditions holding on the days when that amplitude occurs.

A general relationship seems to exist between soil amplitude and rainfall, an increase in amplitude being associated with low average

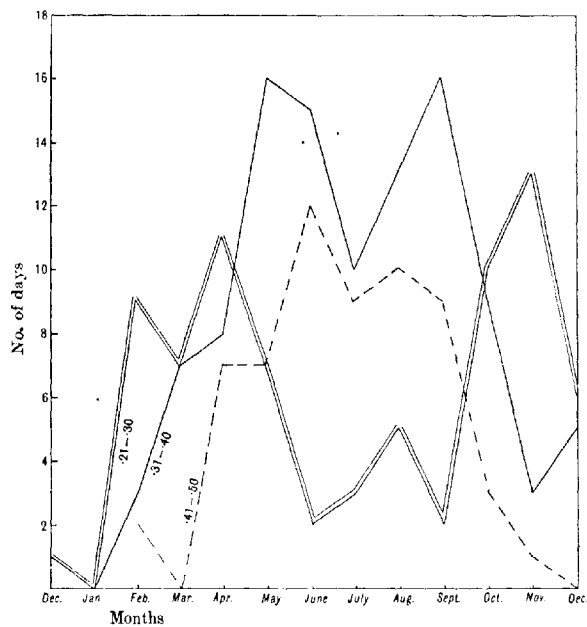


Fig. 7. Monthly distribution of given values of the ratio:  $\frac{\text{soil amplitude}}{\text{air amplitude}}$  [cooling amplitudes].

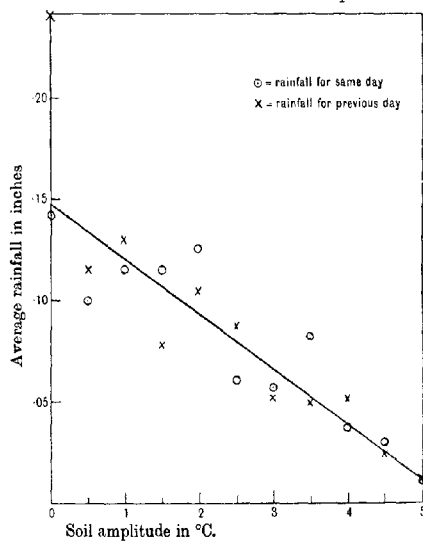


Fig. 8. Relation between soil amplitude and average rainfall for each amplitude.

rainfall and vice versa. This is shown by the fact that the values of amplitude when plotted against rainfall fall roughly on a straight line. The data, however, are not entirely coterminous since the whole of the day's rainfall does not necessarily come during the period when the soil is being heated. Fig. 8 shows two sets of rainfall data—for the day itself and for the preceding day respectively: both show the relationship indicated above.

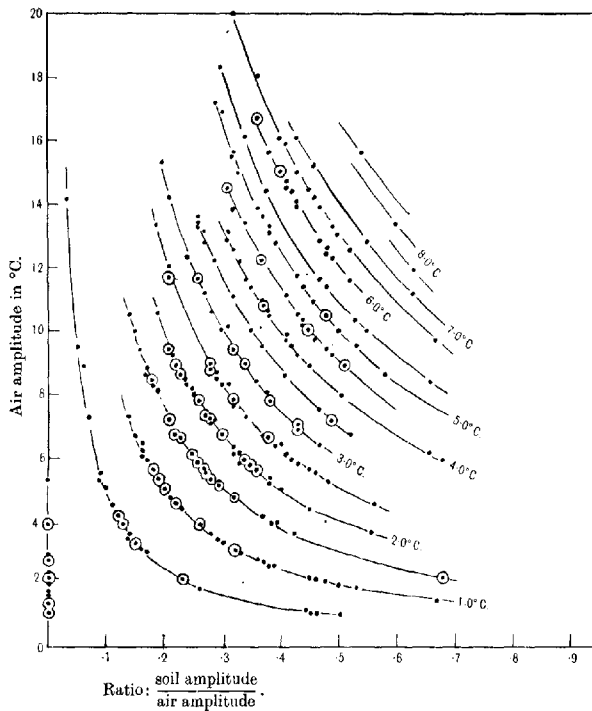


Fig. 9. Relation between air amplitude and the ratio  $\frac{\text{soil amplitude}}{\text{air amplitude}}$ .

(A circle surrounding a dot indicates that that value occurs more than once.)

On the other hand, no relationship could be obtained between rainfall and days when for a given soil amplitude the air amplitude exceeded the average or those where it fell below the average.

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The relationships between conditions and temperature amplitudes are however determined in the main by soil moisture; the influence of the various factors on the amplitude is therefore probably only indirect, the direct action being on the moisture.

Some interesting results appear on plotting the air amplitude against the corresponding ratio  $\frac{\text{soil amplitude}}{\text{air amplitude}}$  (Fig. 9). The points fall within a broad band passing through the origin, which indicates that as the air

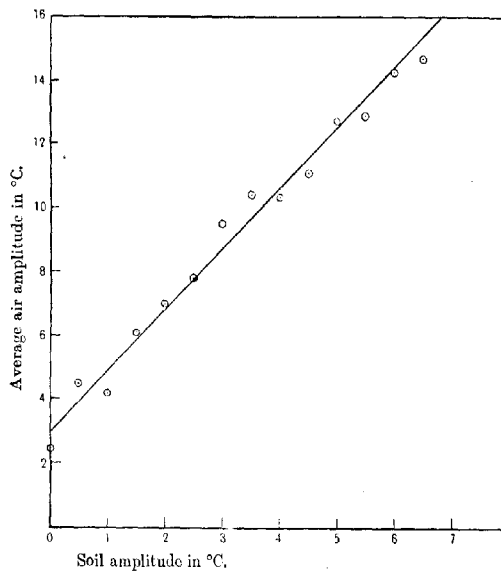


Fig. 10. Relation between soil amplitude and average air amplitude.

amplitude increases the value of the ratio also increases. Closer examination shows that the points lie on a series of hyperbolæ, each of which corresponds to a given soil amplitude. The product of the coördinates for any one hyperbola is

$$\frac{\text{soil amplitude}}{\text{air amplitude}} \times \text{air amplitude} = \text{soil amplitude, i.e. a constant.}$$

These curves represent therefore a series of isothermals of soil temperature amplitudes. For each soil amplitude the curve shows that there is a wide range of possible air amplitudes, mainly because the varying

moisture content of the soil profoundly modifies its thermal properties. If each of these ranges of air amplitude is averaged and plotted against the corresponding soil amplitude the points fall nearly on a straight line, i.e. the relationship between the values is linear (Fig. 10). The values above 6.5° C. are omitted owing to their paucity in numbers. The cutting of the axis by the curve at 3° C. indicates that on the average—there are of course exceptions—an amplitude of air temperature of 3° C. is inappreciable at 6" depth.

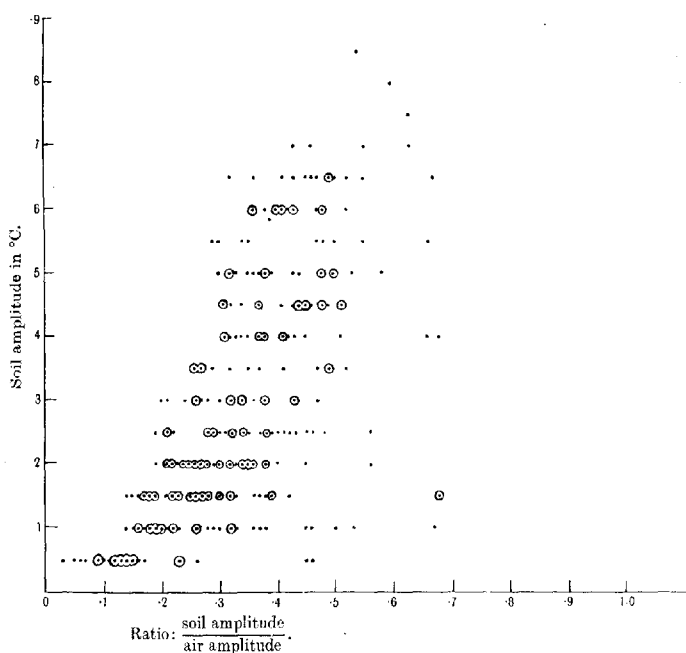


Fig. 11. Relation between soil amplitude and the ratio  $\frac{\text{soil amplitude}}{\text{air amplitude}}$ .

(A circle surrounding a point indicates that that value occurs more than once.)

Fig. 11 shows that the ratio  $\frac{\text{soil amplitude}}{\text{air amplitude}}$  also increases when the soil amplitude increases. At first sight it would hardly be expected that the ratio should increase when either its numerator or denominator increases, but the results show that this is the case. It is due to the fact

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that the soil amplitude increases at a proportionally faster rate than the air amplitude.

### SUMMARY AND CONCLUSIONS.

1. The temperature readings discussed here were taken on a continuous recording soil thermometer at a depth of 6" in the bare soil at Rothamsted.

2. The curves obtained in summer differ from those obtained in winter. In winter there is practically no variation during the day, though fluctuations may extend over a several day period, the maximum of one day being the minimum of the next. In summer there is a marked daily variation; the rise of the temperature which begins on the surface at daybreak penetrates to the 6" depth at about 9.30 a.m. The rise is then continuous till about 4.30 p.m., when it slackens or ceases; after 5.30 p.m. there is a fall which is much slower than the rise, lasting till 8 a.m. or thereabouts. Thus, the temperature rises for about 7 or 8 hours and falls for about 15 or 16 hours, there being often a flat period of varying duration at each end. The mean temperature is passed about mid-day and again at about midnight; the warm period in the soil is thus from midday to midnight, and the cool period from midnight to midday. The soil maximum at 6" is attained about 3 hours later than the air maximum in the screen.

3. The maximum soil temperature is not very closely correlated with number of hours of sunshine, the coefficient for the whole period Feb. 2-Dec. 23 being  $.434 \pm .032$ . The coefficient is even less (viz.  $.357$ ) when account is taken only of the hours of sunshine up to 12.30 p.m. which alone would be expected definitely to influence the maximum soil temperature.

4. On the other hand the extent of the temperature rise at about 6" depth, i.e. the soil amplitude, is closely correlated with the amount of solar radiation as recorded by the Wilson radiointegrator (correlation coefficient =  $+.877 \pm .009$ ) and to a less extent (correlation coefficient =  $+.768 \pm .015$ ) with the hours of sunshine.

5. The extent of the daily rise is adversely affected by rainfall, but apparently not by wind. We found no evidence of rainfall increasing the maximum temperature.

6. The surface of the soil rises to a temperature considerably above that of the air. At 6" depth, however, the temperature wave has been so damped that the maximum is approximately the same as in the air during summer, though it is about  $3^{\circ}\text{C.}$  lower in winter.

7. The cooling of the soil, which determines its minimum temperature, is increased by clear nights but is retarded during autumn by rainfall: during this period rainfall prevents the soil from cooling as much as it otherwise would do.

8. The cooling of the soil does not usually proceed as far as the cooling of the air. In summer the soil minimum may be  $6^{\circ}$  or  $8^{\circ}$  C. above the air minimum, and in winter it is  $3^{\circ}$  C. or more above. Further, the minimum temperature is attained earlier in summer (7.45 a.m.) than in winter (10.30 a.m.), whereas in the air the minimum is attained at approximately the same time (3.45 a.m.) all the year round.

9. The maximum temperature at 6" depth during the summer was usually about  $22^{\circ}$  C. though it rose to  $26.5^{\circ}$  C. on one occasion; the minimum was about  $18^{\circ}$  C., rising to  $21^{\circ}$  C. The mean temperature was about  $20^{\circ}$  C. This mean value was found to show less relationship to radiometer or sunshine values than did the maximum temperature, but there was a traceable connection. The amount of soil moisture, however, showed a tolerably clear connection; the data are not strictly comparable, but it is clear that as the soil dried so it warmed, and as it became moist so it cooled. The transition from winter to summer values takes place quickly—the results of a concurrent increase in soil dryness and in solar radiation. In early winter the cooling of the soil is also rapid, the result probably of fine October nights when radiation is considerable; rainfall retards the process of cooling.

10. It thus appears that the soil population enjoys a warmer climate than that of the atmosphere; it is also moister. The conditions in the surface soil during summer resemble those of a  $20^{\circ}$  C. incubator.

11. Attempts have been made to find substitutes for our admittedly elaborate instrument. The ordinary 12" soil thermometer when used alone proved unsatisfactory: its readings corresponded with the minimum, but not with the mean temperature. The air thermometer readings by themselves proved insufficient, although the soil mean temperature is often about  $3^{\circ}$  C. above the air mean temperature. A fairly satisfactory value for the mean soil temperature is attained by regarding the maximum air temperature as the maximum soil temperature and the 12" soil temperature at 9 a.m. as the minimum, then taking the mean.

12. An increase in either the hours of sunshine or the radiometer values results in an increase in the ratio  $\frac{\text{soil amplitude}}{\text{air amplitude}}$ .

If the soil amplitude increases so does the air amplitude.



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An increase in the soil amplitude is associated with an increase in the ratio  $\frac{\text{soil amplitude}}{\text{air amplitude}}$ .

An increase in the air amplitude is associated with an increase in the ratio  $\frac{\text{soil amplitude}}{\text{air amplitude}}$ .

### APPENDIX I.

#### DESCRIPTION OF INSTRUMENTS.

##### (a) *The 6" Soil Thermometer* (Fig. 12).

This instrument was designed and set up by Sir A. D. Hall when he was Director of Rothamsted Experimental Station. It was put in its present position several years before the period discussed in the text.

Its operation depends on the fact that the linear coefficient of expansion of zinc is greater than that of iron. A zinc rod (*Z*) about 12 ft. long<sup>1</sup> and  $\frac{1}{2}$  inch diameter is enclosed in an iron tube (*I*)  $1\frac{1}{4}$  ins. external diameter, and firmly attached to it at one end. The other end of the zinc rod passes through an oiled collar-bearing at the remaining end of the iron tube, projects five inches beyond it, and carries two brackets (*B*, *B*). A flexible linked steel chain (*cc*)—similar to that on the change-speed-gear of a bicycle—passes from one bracket, once round a spiral groove cut in a metal drum (*d*) 1 inch in diameter, to the other bracket, and is kept taut by nuts on the outer side of each bracket. This drum is carried by an extension arm (*SS*) of the iron tube (*I*), and is free to revolve about a horizontal axis at right angles to the axis of the thermometer. The amount of revolution is controlled by the steel chain: since zinc has a greater coefficient of linear expansion than iron, the projecting portion of the zinc rod moves, relatively to the iron tube, to the right when the 6" depth soil temperature is rising, and to the left when it is falling: hence the resulting linear motion of the steel chain gives a rotary motion to the drum (*d*). In order to prevent any slip of the chain in its spiral groove the centre link is fixed to the drum by a screw. The rotary motion of the drum is magnified by the pointer arm (*P*), 2 ft. long, and the trace is recorded on the clockwork driven drum (*D*) carried by the upper end of the extension arm (*SS*). *W* is a counterpoise weight.

<sup>1</sup> The precise length cannot now be determined without digging up the instrument which, for obvious reasons, we do not wish to do.

Since the clockwork drum is carried by an extension of the iron tube enclosing the zinc rod, it will be seen that the readings are not affected by slight changes in the position of the instrument due to settling of the surrounding soil, etc.

The mechanism is contained in a bricked pit through one side of which the iron tube of the thermometer passes.

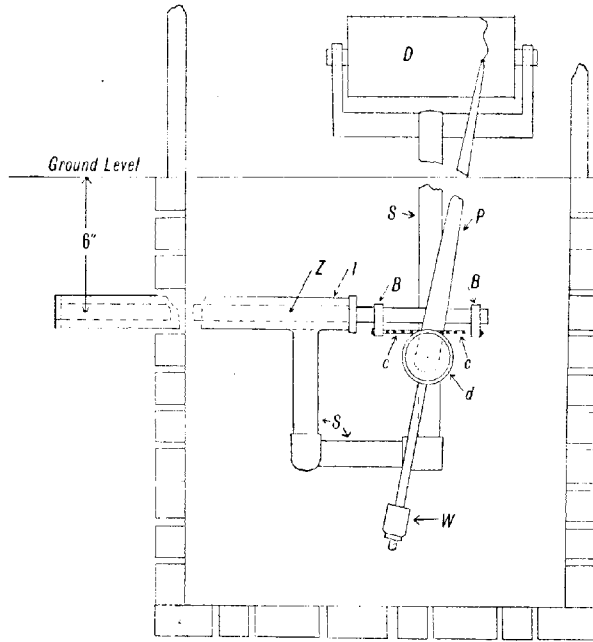


Fig. 12. Diagram illustrating recording mechanism of 6" soil thermometer.

It is possible to calculate the lateral movement of the pen for a known change in temperature from the dimensions of the instrument; the actual calibration however was done by direct comparison with mercury thermometers. This was necessary because of the possibility that an appreciable time-lag might occur before the zinc rod attained the same temperature as its iron sheath. The thermometers used were selected ones of the ordinary "factory" pattern, in which the  $-10^{\circ}\text{C}$ . graduation was about 7" from the bulb. Three of these, after calibration against a standard thermometer, were put in the soil as close as possible to the soil

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thermometer, the centre of each bulb being 6" below ground level. One thermometer was placed near each end of the iron tube, and one in the middle. Calibration was carried out during a warm day in the summer of 1913 after the mercury thermometers had been in position for several months. Readings were taken for 24 hours at approximately two-hourly intervals. In addition to this series a daily reading of the mercury thermometer is always taken at 9 a.m. and compared with the soil thermometer<sup>1</sup>. It was found that the two methods were in good agreement and that there was no evidence of the time-lag mentioned above, doubtless because the fluctuations of soil temperature always take place comparatively slowly. The movement of the pointer corresponding to a change in soil temperature of 1° C. was very nearly 3 mm. It was not easy to read off differences of less than 1·5 mm., so that, as stated in the text, the soil thermometer was read to the nearest 0·5° C. The error due to time-lag in the instrument is therefore well within this limit.

It should be noted that the necessity for having a long zinc rod and iron tube in order to obtain fairly large changes of length between these metals, carries with it the advantage that local irregularities in soil temperature at the given depth are effectively averaged up.

### (b) *The 12" Soil Thermometer.*

This is the familiar Meteorological Office pattern.

An iron tube is driven into the soil to the required depth; a mercury thermometer, enclosed in a glass tube which is attached by a chain to the underside of a copper cap, is lowered into the tube. The chain is of such a length that when the copper cap is in position on the iron tube, the centre of the thermometer bulb is at the 12" depth. In order to prevent the reading of the thermometer changing when it is withdrawn to be read the space between the bulb and the enclosing glass tube is filled with paraffin wax, which is a poor conductor of heat.

### (c) *The Air Thermometer.*

This instrument has a curved capsule containing a liquid with a high coefficient of expansion. One end of the capsule is fixed, and the other free to move. The volume changes of the liquid due to temperature fluctuation cause the capsule to alter its shape. The corresponding

<sup>1</sup> It was found very convenient to record the 9 a.m. reading of the soil thermometer by moving the pointer sideways very slightly with the finger, so as to leave a small mark on the paper. The chart could then be examined at leisure, and the mark, being made at a known time, also afforded a check on the accuracy of the clockwork mechanism.

motion of the free end of the capsule is magnified by levers to readable dimensions, and recorded on the rotatory drum by the pen.

The instrument is enclosed 5 ft. above the ground in a Stevenson screen. The screen has slatted sides, hence the thermometer, being protected from direct sunlight, but open to the atmosphere, records the temperature of the air.

(d) *The Wilson Radio-integrator.*

This instrument measures the total heat radiation reaching the ground by the amount of blackened alcohol vaporised from a glass bulb. Reference to Fig. 13 will explain its action. The top bulb is half-filled with blackened alcohol, so that the surface from which evaporation takes place remains tolerably constant. With the exception of the top hemisphere of the reservoir, the instrument is enclosed in a wooden case and protected from radiation. The vaporised alcohol condenses in the graduated tube and its volume is taken as a measure of the radiation since the time of the last reading. The instrument is set for the next reading by inverting it, thus allowing the alcohol in the graduated tube to run back to the bulb.

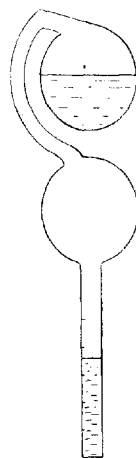


Fig. 13. The Wilson Radio-integrator.

(e) *The Campbell-Stokes Sunshine Recorder.*

This is the familiar Meteorological Office pattern and needs very little description. The sun's rays are focussed by a solid glass sphere into a curved and graduated strip of paper, and thus burn a trace, the total length of which gives the number of hours sunshine during the day. The graduations on the paper correspond to intervals of one hour and thus enable one to read off the duration of sunshine in each hour.

## APPENDIX II (Tables).

Table I. *Weekly means of daily readings.*

Week beginning	AIR THERMOMETER						6" SOIL THERMOMETER							
	Minimum temperature °C.	Maximum temperature °C.	Amplitude of rise °C.	Amplitude of fall °C.	Time of minimum	Time of maximum	Minimum temperature °C.	Maximum temperature °C.	Amplitude of rise °C.	Amplitude of fall °C.	Time of minimum	Time of maximum	Mean temp. (average of min. & max.) °C.	
1913														
Dec. 22nd	0.4	4.9	4.5	4.45	a.m.	p.m.	—	—	—	—	—	—	—	
29th	-0.7	4.0	4.7	3.4	6.30	2.30	—	—	—	—	—	—	—	
1914														
Jan. 5th	2.7	6.7	4.0	5.0	12.30	2.0	—	—	—	—	—	—	—	
12th	-0.3	1.9	2.15	1.9	2.0	4.30	—	—	—	—	—	—	—	
19th	-1.8	3.1	4.95	4.3	5.0	2.30	—	—	—	—	—	—	—	
26th	5.1	8.6	3.5	3.9	2.0	1.30	3.1	4.4	—	—	—	—	—	
Feb. 2nd	3.7	10.3	6.6	6.9	2.30	1.30	4.0	5.3	1.3	2.0	p.m. 12.30	p.m. 6.30	4.65	
9th	5.6	10.6	5.0	5.1	2.30	1.30	5.8	7.5	1.7	1.7	—	6.30	6.65	
16th	1.2	7.8	6.6	7.0	4.0	3.30	3.6	5.1	1.5	2.2	Noon	7.30	4.35	
23rd	0.8	9.0	8.2	8.2	5.30	1.0	2.9	5.0	2.1	1.9	a.m. 10.30	5.0	3.95	
Mar. 2nd	4.5	9.4	4.9	3.6	3.0	1.30	5.1	6.5	1.4	1.7	9.30	5.0	5.8	
9th	3.0	9.8	6.8	6.7	3.30	2.30	4.5	6.3	1.8	1.5	9.0	4.0	5.4	
16th	0.3	7.1	6.8	7.3	3.30	2.30	3.4	5.2	1.8	2.3	11.30	5.0	4.3	
23rd	1.8	9.6	7.8	7.6	4.30	1.30	3.7	6.0	2.3	1.8	10.30	4.30	4.85	
30th	5.9	13.9	8.0	7.7	2.30	2.0	7.1	9.6	2.5	2.5	9.30	6.0	8.35	
April 6th	4.2	12.7	8.5	9.1	6.30	3.0	6.6	9.1	2.5	2.6	9.0	6.0	7.85	
13th	3.3	14.9	11.6	11.6	5.30	2.30	6.9	11.6	4.7	4.5	9.30	6.0	9.25	
20th	5.2	17.3	12.1	12.1	4.30	3.0	9.6	13.2	3.6	3.5	9.30	6.0	11.4	
27th	4.1	15.2	11.1	10.0	4.0	2.0	9.7	13.6	3.9	3.7	9.30	7.0	11.65	
May 4th	7.6	12.7	5.1	6.0	3.30	1.30	10.1	11.8	1.7	1.9	9.30	6.0	10.95	
11th	6.8	15.9	9.1	9.1	2.30	3.30	10.3	14.4	4.1	3.4	8.30	5.30	12.35	
18th	8.0	20.7	12.7	12.8	4.0	2.30	14.3	19.1	4.8	4.9	8.0	5.30	16.7	
25th	4.9	15.1	10.2	10.0	3.30	1.30	11.3	15.2	3.9	3.8	7.30	4.30	13.25	
June 1st	7.1	16.7	9.6	9.5	3.30	2.30	13.6	17.0	3.4	3.6	8.0	5.30	15.3	
8th	8.6	19.2	9.6	8.8	2.0	1.30	13.1	17.3	4.2	3.5	8.30	6.0	15.2	
15th	10.1	22.0	11.9	12.0	4.0	3.0	16.9	21.8	4.9	4.8	8.30	6.0	19.35	
22nd	9.2	21.6	12.4	11.7	3.30	2.30	16.8	22.2	5.4	5.0	7.30	6.0	19.5	
29th	12.0	23.4	11.4	11.3	3.0	1.30	19.4	24.9	4.6	4.1	8.0	5.0	21.7	
July 6th	11.1	21.6	10.5	10.1	3.30	4.0	17.2	21.7	4.5	4.1	7.30	5.30	19.45	
13th	10.9	20.8	9.9	9.6	3.0	2.30	19.2	23.3	4.1	4.0	8.0	5.30	21.25	
20th	12.4	19.3	6.9	7.1	3.30	12.30	17.7	20.2	2.5	3.1	8.30	6.0	18.95	
27th	10.5	18.6	8.1	7.8	1.30	2.30	16.0	19.0	3.0	2.9	7.30	5.30	17.5	
Aug. 3rd	10.3	18.7	8.4	7.8	4.30	2.30	16.6	19.4	2.8	3.2	8.0	5.30	18.0	
10th	12.8	23.0	10.2	10.8	4.0	2.30	17.2	21.9	4.7	4.7	9.0	6.0	19.65	
17th	10.6	21.0	10.4	10.0	3.30	3.0	16.3	20.6	4.3	4.1	9.0	5.30	18.45	
24th	12.1	22.9	10.8	10.9	4.0	3.0	17.1	21.2	4.1	4.1	8.30	5.30	19.15	
31st	11.2	23.3	12.1	12.4	5.0	3.0	16.9	21.6	4.7	5.1	8.0	5.30	19.25	
Sept. 7th	11.6	24.2	12.6	9.7	3.0	2.0	15.9	18.4	2.5	3.3	8.30	4.0	17.15	
14th	8.8	15.9	7.1	8.0	4.30	1.30	12.7	15.3	2.6	3.1	8.30	4.30	14.9	
21st	3.6	17.2	13.6	13.0	5.30	2.30	10.1	15.0	4.9	4.6	8.30	5.30	12.55	
28th	6.5	14.9	8.4	9.5	3.30	2.30	11.1	14.1	3.3	3.2	9.0	5.0	12.75	
Oct. 5th	6.9	14.4	7.5	8.6	3.0	12.30	11.0	13.3	2.3	2.6	9.30	3.30	12.15	
12th	6.8	13.1	6.3	5.3	6.0	2.30	10.2	11.8	1.6	1.6	9.0	4.0	11.0	
19th	7.4	13.1	5.9	5.3	2.30	2.0	9.9	11.4	1.5	1.4	10.0	3.30	10.65	
26th	5.5	11.7	6.2	6.5	3.0	1.0	8.5	10.0	1.5	2.1	9.30	3.30	9.25	
Nov. 2nd	6.2	12.8	6.6	6.5	6.0	2.0	8.1	10.1	1.7	2.4	7.30	3.30	9.25	
9th	4.6	11.1	6.5	7.2	2.30	2.0	7.0	8.3	1.3	1.7	9.30	4.30	7.65	
16th	0.4	4.5	4.1	4.7	4.30	2.30	3.0	3.5	0.5	1.3	11.0	9.30	3.25	
23rd	2.2	7.8	5.6	4.4	4.30	2.0	3.4	5.1	1.7	1.1	7.30	3.30	4.25	
30th	4.7	7.6	2.9	5.1	4.0	3.30	5.6	7.0	1.4	1.3	8.0	7.0	6.3	
Dec. 7th	4.3	7.3	3.0	3.2	3.30	2.0	4.6	5.6	1.0	1.7	9.0	5.30	5.1	
14th	2.8	6.2	3.4	4.1	3.30	2.30	4.0	5.5	1.5	1.7	10.0	7.30	4.75	
21st	0.0	4.3	4.3	3.2	1.30	3.0	1.7	2.2	0.5	0.75	—	—	1.95	

Table II. *Frequency values for the ratio  $\frac{\text{soil amplitude}}{\text{air amplitude}}$ .*

Ratio	No. of times occurring		Ratio	No. of times occurring	
	Temp. rise	Temp. fall		Temp. rise	Temp. fall
.00-.05	2	2	.51-.55	12	6
.06-.10	4	7	.56-.60	4	3
.11-.15	15	15	.61-.65	2	1
.16-.20	25	11	.66-.70	7	—
.21-.25	33	30	.71-.75	—	1
.26-.30	58	46	.76-.80	—	—
.31-.35	42	43	.81-.85	—	1
.36-.40	43	65	.86-.90	—	—
.41-.45	36	40	.91-.95	—	1
.46-.50	28	20	.96-1.01	1	—

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# ON THE USE OF TAKA-DIASTASE IN ESTIMATING STARCH.

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IN an attempt to estimate starch in the grain of wheat grown under varying manurial conditions the writer has obtained results which it seems desirable to put on record for the information of others who may be using or propose to use the Taka-diastrase method<sup>1</sup>.

Before devoting attention to wheat, experiments were made with pure potato starch under the conditions advocated by Davis and Daish. At first, owing to the use of insufficient enzyme, the results were low but immediately 0.1 gm. was taken the results were satisfactory (Table I).

Table I.

Vacuum dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
				grams	per cent.
2.0526	1.4866	0.5645	2.63	2.0511	99.93
2.0562	1.3134	0.9305	1.41	2.0640	100.38
2.4642	2.1524	0.5410	3.98	2.4199	99.42

The wheat examined had been grown under different manurial conditions on Broadbalk Field in 1911. The estimation of starch in the grain involves no less than seven distinct operations, namely (1) sampling and grinding, (2) drying the weighed sample in vacuo, (3) washing out the sugars and amylans, (4) gelatinising the starch, (5) hydrolysing the gelatinised starch solution with taka-diastrase, (6) defecating the hydrolysed starch solution with basic lead acetate solution and removing the excess of lead, and (7) observing the optical rotation and estimating the cupric reducing power of the de-leaded solution; it is therefore not surprising that there was considerable difficulty in getting duplicate analyses to agree. The operation of washing the wheat was undoubtedly the cause of the large discrepancies which were often observed, but after numerous analyses had been made, conditions of washing were evolved which produced concordance in the duplicate results. Figures were obtained such as (1) plot 15, 57.70 and 57.70, (2) plot 19, 58.59 and 58.51. It was

<sup>1</sup> Davis and Daish, *Journ. Agric. Sci.* 1914, 6, p. 151.

noticed however that such results although agreeing with each other were lower than some previously obtained with the same samples. At first, this fact was disregarded—it is more natural to place reliance upon duplicate results which agree than upon those which do not—but when this phenomenon persisted as shown by Table II the suspicion was

Table II.

Wheat from	Earlier results	Later results
Plot 15...	59.29	57.70
	58.51	57.70
Plot 19...	60.26	58.59
	59.81	58.51
Plot 7 ...	59.98	59.36
	59.50	59.24
Plot 10...	60.73	59.22
	60.04	59.15
Plot 2 A	59.79	57.59
	58.30	57.62
Plot 11...	62.34	59.48
	62.06	59.38
Plot 20...	60.37	58.97
	59.64	59.29

aroused that hydrolysis of the starch was incomplete (involving persistence of dextrin, which having no reducing action on Fehling solution and being co-precipitated by basic lead acetate solution would cause the result to be low). To test this question, the solutions from a pair of analyses which had been carried as far as the hydrolysis with taka-diastrase were used. Treatment of the first solution was completed in the usual way, the second was digested for 20 hours at 38° with an additional 0.1 gram of enzyme and the estimation completed as in the first experiment. The results obtained, shown in Table III, prove that 0.1 gram of enzyme was, in fact, insufficient.

Table III.

Taka-diastrase grams	Vacuum-dried Wheat (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
					grams	per cent.
0.1	4.4058	1.7301	1.0441	1.66	2.5468	57.80
0.2	4.4102	2.3734	0.5799	4.09	2.6857	60.92

Most enzymes do not produce a complete change in the substrate, but the hydrolysis of cane-sugar by invertase and of urea by urease are notable exceptions to this rule, and from the work of Davis and Daish it seemed that the action of taka-diastrase on gelatinised starch was another. Accordingly it was argued that, as 0.1 gram of enzyme effected the hydrolysis of nearly 95 per cent. of the starch present in wheat,



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0.2 gram should cause complete hydrolysis to maltose and dextrose. So analyses of eleven samples of wheat were made using 0.2 gram of taka-diastase. The duplicate results agreed satisfactorily, the percentages of starch in every case being higher than in any previous analyses of the same samples. A noteworthy observation was that in these experiments the ratio of dextrose to maltose was far higher than had ever been noticed in former analyses. These experiments exhausted the sample of taka-diastase which had hitherto been used, and when resort was made to another sample, using 0.2 gram for each hydrolysis, the results were again low and the dextrose : maltose ratio fell. Experiments were therefore made with still larger quantities of enzyme, with the results given in Table IV.

Table IV.

Wheat from	Enzyme grams	Vacuum-dried Wheat (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
						grams	per cent.
Plot 8	0.2	4.3610	2.1287	0.7011	3.03	2.5804	59.16
	0.4	4.3590	2.3738	0.5286	4.49	2.6385	60.53
Plot 9	0.4	4.3492	2.5289	0.5279	4.79	2.7745	63.79
	0.6	3.7578	2.3533	0.3278	7.18	2.4287	64.63
Plot 10	0.4	4.3592	2.5353	0.4679	5.42	2.7252	62.52
	0.6	4.3576	2.5953	0.4294	6.04	2.7428	62.94

These results rendered evident the necessity for further experiments with pure starch. Before giving an account of these it will perhaps be desirable to describe in detail the technique followed. The pure ("microscopically tested") potato starch was weighed into a stoppered flask, such as is described by Davis, Daish and Sawyer<sup>1</sup>, and dried by heating the flask in a Victor Meyer vapour bath at 110° whilst connected to another flask containing phosphorus pentoxide and evacuated by means of a good water-pump. Heating for 8-9 hours was generally sufficient. The dry starch was washed into a 400 c.m. Phillips conical beaker with 200-250 c.cm. of water and gelatinised by immersing the beaker in boiling water for half an hour, the starch and water being continually stirred during the first five minutes to prevent "balling." Any starch-jelly which adhered to the walls of the beaker above the level of the liquid was rubbed down with a glass rod, the solution cooled to 38°, covered with a layer of toluene and the weighed amount of dry taka-diastase washed in with a small volume of water. The beaker was placed in a thermostat at 38° for 16-24 hours (in the initial experiments the time of hydrolysis was 18 hours, but latterly 24 hours has been the duration) and then the enzyme inactivated by immersing the beaker in boiling water for 15

<sup>1</sup> *Journ. Agric. Sci.* 1916, **7**, p. 269, Fig. 2.

minutes. The hydrolysed starch solution was washed into a 500 c.cm. graduated flask containing 0.5 gram of sodium fluoride (when the solution resulting from the hydrolysis of pure starch with taka-diastase is treated with basic lead acetate solution an opalescence is formed which cannot be removed by filtration, so a small quantity of sodium fluoride was added in order to produce a coagulable precipitate), cooled to 15°, and treated with basic lead acetate solution—4 c.cm. being the volume usually added. The liquid was diluted to exactly 500 c.cm. and filtered as quickly as possible through a dry filter. Two "sugar" flasks, graduated both at 100 and 110 c.cm., were filled to the 100 c.cm. mark with the filtrate and the excess of lead removed by adding small amounts of powdered anhydrous sodium carbonate until a fresh addition produced no further cloudiness. The two solutions were then diluted to 110 c.cm. (at 15°) and filtered through dry filters. The optical rotatory powers of the filtrates were determined at 20° in a 400 mm. tube using sodium light, and the cupric reducing power of 25 c.cm. of each filtrate was estimated under the conditions described by Brown, Morris and Millar<sup>1</sup>. From these optical rotations and weights of copper oxide the weight of starch was calculated as in the example given by Davis and Daish<sup>2</sup>.

In the first instance six experiments were made with either 0.2 or 0.4 gram of a sample of taka-diastase 2½ years old, hydrolysis being continued for 18 hours in the first four experiments and for about 40 hours in the last two. The results were as follows:

Table V.

Exp.	Enzyme grains	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
						grams	per cent.
1	0.2	3.2610	1.7362	1.5978	1.09	3.0771	94.36
2	0.2	3.2590	1.8568	1.4617	1.27	3.0566	93.78
3	0.4	3.2564	2.0469	1.2221	1.67	3.0006	92.14
4	0.4	3.2586	2.0271	1.2932	1.57	3.0501	93.60
5	0.4	2.8388	2.3008	0.6357	3.62	2.6733	94.17
6	0.4	2.8378	2.5940	0.4329	5.99	2.7450	96.73

A characteristic feature of these results is the low value of the dextrose : maltose ratio in the first four experiments. It was generally though not invariably found that the higher the value of this ratio the better the result.

For the next six experiments a new sample of enzyme, received from Messrs Parke, Davis and Co. only three months previously, was used. In experiments numbered 7, 8, 9, and 10 hydrolysis was effected in one operation lasting 18 hours, but in the eleventh experiment 0.1 gram of

<sup>1</sup> *Chem. Soc. Trans.* 1897, **71**, 100.<sup>2</sup> *Journ. Agric. Sci* 1914, **6**, pp. 160-61.

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the enzyme was added initially, then a further 0.1 gram after 18 hours and the hydrolysis continued for another 24 hours. In experiment no. 12 four quantities each of 0.1 gram of the taka-diastase were added after 0 hours, 18 hours, 42 hours and 66 hours respectively, the total duration of the hydrolysis being 90 hours.

Table VI.

Exp.	Enzyme grams	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
						grams	per cent.
7	0.1	2.8344	0.9654	1.9276	0.50	2.6959	95.12
8	0.1	2.8328	0.7913	2.0926	0.38	2.6957	95.16
9	0.2	2.8330	0.6789	2.1248	0.32	2.6250	92.66
10	0.2	2.8326	0.6459	2.1974	0.29	2.6641	94.05
11	0.2	2.8390	0.9937	1.8517	0.53	2.6495	93.33
12	0.4	2.8394	1.9166	0.9572	2.00	2.6323	92.70

Thus the results obtained with a fresh sample of enzyme were no better than with that  $2\frac{1}{2}$  years old. Next two experiments were made with a third sample of taka-diastase received eighteen months previously. A new sample of pure potato starch was here used and the hydrolysis was continued for 24 hours. In the second of these two experiments part of the hydrolysed starch solution was merely filtered before the determination of the optical rotatory power and cupric reducing power (figures for which are given in the second line of the Table below), whilst the remainder was treated with basic lead acetate solution as usual (figures in last line of Table).

Table VII.

Exp.	Enzyme grams	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
						grams	per cent.
13	0.2	2.8596	0.9352	1.9362	0.48	2.6770	93.61
14	0.2	2.8640	0.9140	2.0600	0.44	2.7752	96.90
			0.9471	1.9870	0.48	2.7358	95.52

The fact that a higher result was obtained when the solution was merely filtered than when basic lead acetate was used and the observation that the optical rotation of the filtered solution was reduced from  $2^{\circ}412$  to  $2^{\circ}341$  whilst the weight of copper oxide precipitated was only reduced from 0.2338 gram to 0.2332 gram (dextrin is optically active but does not reduce Fehling solution), support the view that unchanged dextrin remains in solution.

The deficiency in the starch-content found might be due to impurity in the sample. To determine this point experiments were made with 0.1 gram of malt diastase. Two analyses, carried out very much as described above but hydrolysing at  $55^{\circ}$  for three hours and of course without the use of basic lead acetate, gave the results in Table VIII.

Table VIII.

Exp.	Vacuum-dried Starch (grams)	Dextrin grams	Maltose grams	Starch	
				grams	per cent.
15	2.0276	0.5084	1.6037	2.0285	100.04
16	1.6478	0.4096	1.3087	1.6501	100.14

Evidently the sample of starch was pure.

In the majority of the above experiments with pure potato starch and taka-diastrase the dextrose : maltose ratio is low, indicating that there was a deficiency of maltase in the enzyme preparation used. It is known that maltose exerts a retarding influence on starch-liquefying enzymes; accordingly the apparently incomplete hydrolysis might be due to the accumulation of maltose in the solution, and it was thought possible that if maltase were added to the taka-diastrase complete hydrolysis might be effected. To ascertain whether such an effect could be produced two experiments were made under identical conditions except that to the first (17 in Table IX) 0.1 gram of taka-diastrase was added and one hour later 50 c.cm. of an extract of yeast prepared by digesting one part of dried brewers' yeast with ten parts of water at 25°, whilst to the second (18 in Table IX) only the taka-diastrase (0.1 gram) was added.

Table IX.

Exp.	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose		Starch	
				Maltose	grams	per cent.	
17	2.8646	1.4223	1.4648	0.97	2.6685	93.15	
18	2.8658	0.4147	2.4108	0.17	2.6678	93.09	

These figures show that although the addition of yeast extract increased the proportion of maltose hydrolysed, it did not increase the amount of starch found.

Failure having attended all efforts to attain success with commercial samples of taka-diastrase, attempts were made to obtain better results with an enzyme prepared in the laboratory. *Aspergillus Oryzae* was grown at 25° on moist sterile bran (contained in eight 800 c.cm. conical flasks) for 20 days. The product in the first flask was digested with water and toluene for about 1-2 hours, then the mass was filtered on a Buchner funnel and the filtrate used to extract the growth in the second flask and so on until all eight flasks had been dealt with. The extract was precipitated with three volumes of 95 per cent. alcohol, the precipitate allowed to settle, filtered on a Buchner funnel, washed with alcohol and dried in vacuo. Of this precipitate 0.1 gram was used in each of two experiments with pure potato starch with the following results:

Table X.

Exp.	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
					grams	per cent.
19	2.8656	1.5165	1.4152	1.07	2.7063	94.44
20	2.8692	1.4755	1.4254	1.03	2.6791	93.38

In further experiments the fungus was grown as before but only for seven days. The extract was precipitated first with 65 per cent. of its volume of alcohol (95 per cent.) and then the filtrate treated with excess of alcohol. An experiment was made with 0.2 gram of each precipitate giving the results recorded in Table XI, Exp. 21 referring to the first precipitate, Exp. 22 to the second.

Table XI.

Exp.	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
					grams	per cent.
21	2.8638	2.2477	0.7039	3.19	2.6901	93.94
22	2.8738	2.0277	0.9761	2.08	2.7501	95.70

These experiments, like the previous ones, were not successful, but it is noticeable that the dextrose : maltose ratio is much higher than in the case when the fungus was three weeks old.

The fungus was next grown on Munter's medium (dipotassium hydrogen phosphate 1 gram, crystallised magnesium sulphate 0.5 gram, nitrogen as ammonium chloride 0.1 gram, starch 40 grams, water 1 litre) for seven days, then dried in vacuo, ground in a mill and the fungus used without extraction of the enzyme, 1 gram in the first experiment (23), 1.5 grams in the second (24).

Table XII.

Exp.	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
					grams	per cent.
23	2.8720	0.0148	2.5959	0.006	2.4739	86.14
24	2.8740	0.4283	2.3775	0.18	2.6391	91.82

These results are characterised by an extremely low dextrose : maltose ratio—the lowest so far obtained.

It seemed possible that a fungus grown in the presence of dextrin might secrete more dextrinase and so give a more active product. To ascertain whether such is the case *Aspergillus Oryzae* was cultivated for ten generations on a Munter's medium in which the starch was replaced with dextrin, then a large culture was grown on the same medium for seven days, dried in vacuo and 1 gram used in an experiment (25) with pure potato starch. For Exps. 26 and 27 the fungus was grown on the

same dextrin medium except that it contained asparagin as the source of nitrogen. In Exp. 26, 0.8 gram of a seven-day culture was used, in Exp. 27, 1.0 gram of a fourteen-day culture.

Table XIII.

Exp.	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
					grams	per cent.
25	2.0600	0.7878	1.2976	0.61	1.9389	94.12
26	2.0630	0.7953	1.3277	0.60	1.9743	95.70
27	1.6424	0.3929	1.1903	0.33	1.4817	90.22

It seems therefore that asparagin gives a more active product, but that the efficiency diminishes with continued growth. To obtain information as to the effect of the age of the fungus, another culture was grown on moist bran, but in this case only for four days. The fungus was extracted with water, the extract precipitated with alcohol, the precipitate dried in vacuo and 0.2 gram of it used.

Table XIV.

Exp.	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
					grams	per cent.
28	1.6516	0.5714	1.0562	0.58	1.5154	91.76

Thus the four-day growth, like those of 14 and 20 days, is less active than that cultivated for seven days. In order to ascertain at what stage of growth *Aspergillus Oryzae* has a maximum activity towards starch the following series of experiments was made. Eight 800 c.cm. conical flasks each containing 50 grams of moist bran were sterilised, inoculated each with 5 c.cm. of a suspension of the fungus spores in sterilised water and placed in an incubator at 25°. After 1, 2, 3, 4, 5, 6, 7 and 15 days respectively one of the flasks was removed, 5 c.cm. of toluene added and the mass of bran and fungus extracted with 250 c.cm. of water for one hour at the room temperature. The mass was then filtered on a Buchner funnel and 50 c.cm. of the filtrate used to hydrolyse 2 grams of gelatinised starch for 24 hours at 38°. At the same time 50 c.cm. of the extract were added to about 200 c.cm. of water and the solution kept at 38° for 24 hours for the determination of the optical rotatory power and cupric reducing power of the enzyme extract *per se*. The results obtained are given in Table XV.

The extract from the seven-day growth being apparently the most active of all, another experiment (36 in the Table) was made with it, but using 100 c.cm. instead of 50 c.cm., with the result shown.

The figures given in Table XV seem to show that the activity of

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the fungus towards starch increases up to the fifth day of growth and then diminishes, although the product of seven days' growth is apparently exceptional. There is no doubt however that the fungus contains a maximum amount of maltase after five days' growth.

Table XV.

Exp.	Growth days	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
						grams	per cent.
29	1	1.6430	0.6389*	0.9177	—	1.5088	91.80
30	2	1.6484	0.8263	0.7592	1.09	1.4633	88.80
31	3	1.6406	1.2813	0.3811	3.36	1.5144	92.30
32	4	1.6486	1.5132	0.1873	8.08	1.5395	93.40
33	5	1.6492	1.5801	0.1429	11.06	1.5575	94.40
34	6	1.6484	1.5306	0.1742	8.78	1.5427	93.60
35	7	1.6492	1.5245	0.2113	7.21	1.5723	95.30
36	7	1.6450	1.5216	0.2429	6.26	1.5997	97.20
37	15	1.6406	1.3983	0.2869	4.87	1.5304	93.30

\* Dextrin not dextrose; in this experiment the usual calculation gives a negative amount of dextrose, the figures given are obtained on the assumption that only maltose and dextrin are formed.

Another experiment was made under the conditions of the above series, but two flasks only of moist bran inoculated with the aspergillus spores were kept at 25° for five days and then extracted as above. In the first experiment (38) 50 c.cm. of the extract were used, in the second (39) 150 c.cm.

Table XVI.

Exp.	Extract c.cm.	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
						grams	per cent.
38	50	1.6438	1.4618	0.2433	6.01	1.5462	94.06
39	150	1.6506	1.4831	0.1582	9.37	1.4847	89.90
40	50	1.6446	1.1760	0.4549	2.58	1.4896	90.58

Experiment 39 was made a month after the extract had been first prepared, when it had evidently greatly diminished in activity.

J. Takamine<sup>1</sup> for the preparation of taka-diastase advocates the extraction of a mixture of raw bran and bran upon which the fungus has been grown. For this reason an experiment (40 in Table XVI) contemporary with the first (38) of the above experiments was made under identical conditions except that in addition to the 50 c.cm. of aspergillus extract, 50 c.cm. of an extract of bran (1 part of bran to 4 parts of water + toluene) were added. This gave not a better but a worse result.

A fresh sample of enzyme labelled "Concentrated Taka-diastase free from Lactose" having been received from Messrs Parke, Davis and Co.,

<sup>1</sup> English Patent No. 17,277, A.D. 1894.

some experiments were made with it. The results of three are given in Table XVII.

Table XVII.

Exp.	Enzyme grams	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
						grams	per cent.
41	0.1	1.6498	0.9738	0.7012	1.39	1.5392	93.29
42	1.0	1.6496	1.7020	0.0471	36.13	1.5754	95.60
43	0.1	1.6504	1.3213	0.3738	3.53	1.5435	93.52

In Exp. 41, 0.1 gram of the new taka-diastase (No. 9) was used, in Exp. 42 the same weight of enzyme was added daily to a solution of gelatinised starch contained in a stoppered flask (at 38°) for ten days. Exp. 43 was made in the presence of 0.075 gram of asparagin, which has been stated to accelerate the action of diastase. From the results given in Table XVII the new sample of taka-diastase does not seem to be any more efficient than previous ones.

The whole of the above results having been discussed with Mr W. A. Davis, he suggested firstly that the starch might not have been completely dried—he heated the starch at 120° as against 110° in this work—and secondly that the low values for the apparent starch-content might be due to the destruction of sugars, either by the action of the basic lead acetate or of the excess of sodium carbonate (used to precipitate the excess of lead), rather than to the persistence of dextrin. It was found that two weighed quantities of starch, when dried in vacuo at 130–134°, lost 17.55 and 17.48 per cent. of their weight as against 17.48, 17.47, 17.77 and 17.81 per cent. found in the four previous determinations at 109–110°. To test the question of the destruction of sugars, experiments with pure starch were made as already described, but part of the hydrolysed starch solution was clarified with alumina cream and the remainder with basic lead acetate solution, a portion of the latter being de-leaded at once and the remainder after standing some hours. The excess of lead was in some cases removed with just sufficient sodium carbonate and in others with a considerable excess. The results obtained are given in Table XVIII.

From examination of the figures in Table XVIII it seems evident that the optical rotation given by hydrolysed starch solutions is less after defecation with basic lead acetate solution than after treatment with alumina cream. This cannot be due to a difference in the amounts of dissolved enzyme removed by the two reagents because the figures given have already been corrected by the deduction of the optical rotations and weights of copper oxide given by blank experiments, i.e. taka-diastase



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solutions which were clarified in the same way. It is noticeable however that the difference in the weights of copper oxide given after clarification with alumina cream and after treatment with basic lead acetate solution is proportionately much less than the corresponding difference in the optical rotations. This suggests that a substance (? dextrin) which has a high rotatory power but little or no reducing action on Fehling solution, has been removed (compare Exp. 14 of Table VII). On the other hand the

Table XVIII.

Exp.	Vacuum-dried Starch grams	Clarifying agent	Excess of sodium carbonate	Optical rotation degrees	CuO grams	Dextrose grams	Maltose grams	Starch	
								grams	per cent.
44	2-8856	Alumina cream	—	2-097*	0-2571*	1-3797	1-5670	2-7271	94-51
		Basic lead, excess precipitated after 40 mins. Basic lead, excess precipitated after 23 hrs.	Nil	1-887	0-2316				
			0-005	1-842	0-2351				
			Nil	1-842	0-2312				
			Nil	1-855	0-2332				
45	2-8882	Alumina cream	—	2-229*	0-2496*	1-2267	1-7576	2-7699	95-91
		Basic lead, excess precipitated after 40 mins. Basic lead, excess precipitated after 8 hrs. Ditto, after 44 hrs.	Nil	2-006	0-2266				
			0-075	1-908	0-2284				
			Nil	1-906	0-2286				
			0-300	1-911	0-2268				
			—	1-903	0-2268	1-3054	1-6174	2-7079	93-76
			—	1-891					

\* The dilutions of the solutions clarified with alumina cream and basic lead acetate respectively were different; the figures marked \* represent the *observed* values, those immediately below them are the values calculated for the same dilution as that of the solution clarified with basic lead acetate.

presence of an excess of sodium carbonate as large as 0.2 gram per 100 c.cm. for 23 hours seems to have no effect on the optical rotation or cupric reducing power of the solution, although the optical rotation is reduced by the presence of a larger excess for a longer time. Further experiments were made in which the hydrolysed starch solution was (1) filtered, (2) treated with alumina cream and filtered, (3) treated with basic lead acetate solution and sodium carbonate and filtered, and (4) treated with sodium fluoride, basic lead acetate solution and sodium carbonate and filtered. The results are recorded in Table XIX. In Exps. 46 and 47 the gelatinised starch was hydrolysed with 0.1 gram of the freshest sample (No. 9) of taka-diastase for 24 hours, the hydrolysed solution introduced into a 500 c.cm. measuring flask containing 1 gram of sodium fluoride and diluted to 500 c.cm. From the solution of Exp. 46 three separate 100 c.cm. portions were treated, the first with 10 c.cm.

of water (*a*), the second with 5 c.cm. of alumina cream and water up to 110 c.cm. (*b*) and the third with 1 c.cm. of basic lead acetate solution, sufficient sodium carbonate to precipitate the excess of lead and water up to 110 c.cm. (*d*). All three liquids were then filtered through dry filters. From the solution of Exp. 47, 50 c.cm. were withdrawn and filtered (*a*), 50 c.cm. were withdrawn, treated with 5 c.cm. of alumina cream and filtered (*b*), 50 c.cm. were withdrawn, treated with 0.5 c.cm. of basic lead acetate solution, sufficient sodium carbonate to precipitate the excess of lead and water up to 55 c.cm. and filtered (*d*), and the remaining 350 c.cm. treated with 4 c.cm. of basic lead acetate solution, diluted to 500 c.cm. filtered and the filtrate preserved. One month later 100 c.cm. were treated with just sufficient sodium carbonate to precipitate the excess of lead, diluted to 110 c.cm. and filtered (*e*). In Exps. 48, 50 and 51 the gelatinised starch was hydrolysed (48) for 16 days with 0.5 gram of taka-diasatase (No. 9) added in portions of 0.1 gram, (50) for 24 hours with 0.1 gram of the same enzyme, and (51) for 24 hours with 0.1 gram of a specimen of taka-diasatase received from Mr C. Revis. The solution from each of these experiments was diluted to 500 c.cm. and four 100 c.cm. portions treated, the first with water (*a*), the second with alumina cream (*b*), the third with basic lead acetate solution and sodium carbonate (*c*), and the fourth with sodium fluoride, basic lead acetate solution and sodium carbonate (*d*), each liquid after treatment being diluted to 110 c.cm. and filtered through a dry filter. The gelatinised starch in Exp. 49 was hydrolysed with a mixture of 0.1 gram of taka-diasatase (No. 9) and 0.1 gram of malt diastase. From the resulting solution four 100 c.cm. portions and one 50 c.cm. portion were withdrawn. The last was treated with 5 c.cm. of water and filtered (*a*), the 100 c.cm. portions were treated, the first with alumina cream (*b*), the second with basic lead acetate solution and sodium carbonate (*c*), the third with sodium fluoride, basic lead acetate solution and sodium carbonate (*d*), and the fourth with basic lead acetate solution allowed to stand for 24 hours and then the excess of lead precipitated with sodium carbonate (*e*), each of the four liquids being diluted to 110 c.cm. and filtered through a dry filter.

A merely casual examination of Table XIX reveals several facts. In the first place, addition of malt diastase to the taka-diasatase evidently does not increase the apparent starch content (compare Exps. 49 and 47) although it raises the dextrose : maltose ratio, which is curious since malt diastase contains little or no maltase, secondly addition of consecutive doses of enzyme to the hydrolysing starch solution effects no improve-

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ment. The latter fact was observed previously in Exps. 11, 12 (Table VI) and 42 (Table XVII), in fact in Exps. 11 and 12 the larger amounts of enzyme actually gave a *lower* starch content, which is an argument against the hypothesis that the deficiency in the apparent starch percentage is due to the persistence of dextrin. Thirdly the differences among the figures for one experiment are least in the case of Exp. 51 in which an enzyme preparation of similar date to the first used in this

Table XIX.

Exp.	Vacuum-dried Starch grams	Clarifying agent	Optical rotation degrees	CuO grams	Dextrose grams	Maltose grams	Starch	
							grams	per cent.
46	2-0650	None (a)	1-341	0-2072	1-3292	0-8309	1-9839	96-07
		Alumina cream (b)	1-348	0-2099	1-3534	0-8287	2-0096	97-02
		NaF, basic lead, Na <sub>2</sub> CO <sub>3</sub> (d)	1-300	0-2079	1-3639	0-7767	1-9637	95-09
47	2-0658	None (a) {	1-561*	0-1784*	1-2240	0-9492	2-0013	96-88
			1-422	0-2024				
		Alumina cream (b)	1-409	0-2067	1-2780	0-9185	2-0208	97-82
		NaF, basic lead, Na <sub>2</sub> CO <sub>3</sub> (d)	1-293	0-1985	1-2657	0-8073	1-9043	92-18
		Ditto, stood 1 month (e)	0-8825	0-1388	1-2493	0-7813	1-8649	90-27
48	2-0620	None (a)	0-864	0-2526	2-1738	0-0308	1-9856	96-29
		Alumina cream (b)	0-873	0-2556	2-2007	0-0295	2-0086	97-41
		Basic lead, Na <sub>2</sub> CO <sub>3</sub> (c)	0-817	0-2495	Calculations give a negative amount of maltose			
		NaF, basic lead, Na <sub>2</sub> CO <sub>3</sub> (d)	0-826	0-2502				
49	2-0656	None (a)	1-168	0-2193	1-5701	0-5658	1-9778	95-75
		Alumina cream (b)	1-200	0-2254	1-6357	0-5727	2-0148	97-54
		Basic lead, Na <sub>2</sub> CO <sub>3</sub> (c)	1-153	0-2186	1-5884	0-5488	1-9451	94-16
		NaF, basic lead, Na <sub>2</sub> CO <sub>3</sub> (d)	1-160	0-2181	1-5778	0-5548	1-9459	94-21
		Basic lead for 24 hours (e)	1-150	0-2179	1-5822	0-5432	1-9389	93-86
50	2-0576	None (a)	1-345	0-2023	1-2725	0-8567	1-9572	95-12
		Alumina cream (b)	1-351	0-2004	1-2473	0-8723	1-9494	94-74
		Basic lead, Na <sub>2</sub> CO <sub>3</sub> (c)	1-367	0-2012	1-2461	0-8888	1-9639	95-45
		NaF, basic lead, Na <sub>2</sub> CO <sub>3</sub> (d)	1-298	0-2005	1-2837	0-8064	1-9187	93-25
51	2-0694	None (a)	1-393	0-2043	1-2632	0-9082	1-9977	96-54
		Alumina cream (b)	1-402	0-2066	1-2822	0-9090	2-0164	97-44
		Basic lead, Na <sub>2</sub> CO <sub>3</sub> (c)	1-411	0-2048	1-2569	0-9286	2-0114	97-19
		NaF, basic lead, Na <sub>2</sub> CO <sub>3</sub> (d)	1-388	0-2044	1-2681	0-9013	1-9956	96-43

\* The first figures given in the fourth and fifth columns for Exp. 47 are the *observed* values, those immediately below them are the calculated values for the same dilution as that of (b) and (d).

work (Table I) was employed. Fourthly the apparent starch values are almost invariably higher after using alumina cream than before (Exp. 50 affording the only exception) a fact which is very difficult to understand. In order to render apparent the conclusions to be drawn from the effects of alumina cream and of basic lead acetate, the changes in optical rotation, in weight of copper oxide, in the dextrose : maltose ratio and

in the apparent starch percentage, produced by the clarifying agent, are collected in Tables XX and XXI.

Table XX.

*Effect of Alumina Cream.*

Exp.	Alumina cream	Optical rotation degrees	Copper oxide grams	Dextrose Maltose	Starch per cent.
46	Not used	1.341	0.2072	1.59	96.07
	Used	1.348	0.2099	1.63	97.02
47	Not used	1.422	0.2024	1.29	96.88
	Used	1.409	0.2067	1.39	97.82
48	Not used	0.864	0.2526	70.6	96.29
	Used	0.873	0.2556	74.6	97.41
49	Not used	1.198	0.2193	2.63	95.75
	Used	1.200	0.2254	2.86	97.54
50	Not used	1.345	0.2023	1.48	95.12
	Used	1.351	0.2004	1.43	94.74
51	Not used	1.393	0.2043	1.39	96.54
	Used	1.402	0.2066	1.41	97.44

Table XXI.

*Effect of Sodium fluoride + Basic lead acetate + Sodium carbonate.*

Exp.	Clarifying agent	Optical rotation degrees	Copper oxide grams	Dextrose Maltose	Starch per cent.
46	Not used	1.341	0.2072	1.59	96.07
	Used	1.300	0.2079	1.76	95.09
47	Not used	1.422	0.2024	1.29	96.88
	Used	1.293	0.1985	1.37	92.18
48	Not used	0.864	0.2526	—	—
	Used	0.826	0.2502	—	—
49	Not used	1.198	0.2193	2.63	95.75
	Used	1.160	0.2181	2.84	94.21
50	Not used	1.345	0.2023	1.48	95.12
	Used	1.298	0.2005	1.59	93.25
51	Not used	1.393	0.2043	1.39	96.54
	Used	1.388	0.2044	1.40	96.43

Dextrose has a lower specific rotatory power and a higher cupric reducing power than has maltose. Consequently a maltose solution which is undergoing hydrolysis—and therefore has an increasing dextrose : maltose ratio—has a falling optical rotation and an increasing capacity for precipitating cuprous oxide. If then a hydrolysed starch solution is subjected to such treatment that its optical rotation decreases and its cupric reducing power increases, the dextrose : maltose ratio of the solution will seem to increase; further, either of these factors (fall in rotation or rise in reduction) operating alone will produce a similar apparent change in the dextrose : maltose ratio though of smaller extent. Conversely a rise in optical rotation or a fall in cupric reducing power

or both phenomena together will produce an apparent fall in the dextrose : maltose ratio. Examination of Table XX in the light of these facts discovers what seems to be a contradiction, for in each of the experiments except one (47) an increase in optical rotation is accompanied by an increase in the dextrose : maltose ratio. The change in the optical rotation in every case is small and may be due to the removal (by the alumina cream) of a small quantity of some laevo-rotatory substance. On the other hand there is a more marked increase in the weight of copper oxide precipitated and the effect of this on the calculation of the dextrose : maltose ratio probably outweighs the effect of the small rise in the optical rotation. At the same time it must be admitted that no explanation has yet been discovered for this increase in the cupric reducing power consequent on the treatment with alumina cream. Reduction of the optical rotation or the cupric reducing power by treatment with a clarifying agent can of course be ascribed to the precipitation of sugar or dextrin, and increase in dextro-rotation to the removal of a laevo-rotatory compound, but it is difficult to understand how such a reagent can *increase* the cupric reducing power of the solution. The effect of clarifying with the sodium fluoride + basic lead acetate + sodium carbonate mixture is different (Table XXI). In this case the optical rotation of the solution is invariably diminished, and the weight of copper oxide precipitated is generally reduced also, although in one case it is practically unaltered and in another it is slightly increased. If the action of this mixture of reagents were solely to remove dextrin (apart from the substances which it is intended to remove) there would be a fall in the optical rotation (accompanied by an increase in the dextrose : maltose ratio) but no change in the cupric reducing power. As a diminution in cupric reducing power generally accompanies the fall in rotation, the cause cannot simply be the precipitation of dextrin. Nevertheless in every case the dextrose : maltose ratio is increased, which means that the diminution in rotation has a greater effect than has the fall in reducing power, suggesting that the chief effect of the clarifying agent is the removal of dextrin. If maltose were precipitated both rotation and reducing power of the solution would of course be diminished and the dextrose : maltose ratio increased. That this however is not the explanation of the decrease in the two factors is shown in Table XXII in which are given values for the diminution in the weight of copper oxide precipitated and for the increase in the dextrose : maltose ratio calculated on the assumption that the fall in rotation (Table XXI) is wholly due to the disappearance of maltose. It will be seen that the observed

decrease in the weight of copper oxide precipitated is never as great as the decrease calculated from the fall in rotation, except in one case (48), in which experiment owing to the use of a large quantity of enzyme the calculated weight of maltose present is so small that its complete removal would not reduce the rotation to the extent of  $0^{\circ}.038$ . Had it been assumed that the decreases in rotation shown in Table XXI were due to the disappearance of dextrose the values calculated for the fall in weight of the copper oxide precipitated would have been about five times as great as those given in Table XXII.

Table XXII.

Exp.	Change in rotation degrees	Change in weight of CuO		Change in D/M ratio	
		Observed	Calculated	Observed	Calculated
46	-0.041	+0.0007	-0.0025	1.59 to 1.76	1.59 to 1.68
47	-0.129	-0.0039	-0.0080	1.29 to 1.57	1.29 to 1.49
48	-0.038	-0.0024	-0.0023	—	—
49	-0.038	-0.0012	-0.0024	2.63 to 2.84	2.63 to 2.81
50	-0.047	-0.0018	-0.0029	1.48 to 1.59	1.48 to 1.57
51	-0.005	+0.0001	-0.0003	1.39 to 1.40	1.39 to 1.40

Learning of the difficulty which the author had encountered Mr C. Revis and Mr H. F. E. Hulton were good enough to supply him with specimens of taka-diastase which they had used. Exps. 51 (Table XIX) and 52 (Table XXIII) were made with the enzyme from Mr Revis, 53 and 54 (Table XXIII) with that from Mr Hulton.

Table XXIII.

Exp.	Enzyme grams	Vacuum-dried Starch (grams)	Dextrose grams	Maltose grams	Dextrose Maltose	Starch	
						grams	per cent.
52	0.2	1.6312	1.3196	0.4212	3.13	1.5868	97.28
53	0.1	2.0716	0.7072	1.4624	0.49	2.0227	97.63
54	0.2	2.0808	0.9348	1.2481	0.75	2.0244	97.29

In Exps. 52-54 the solutions were cleared with alumina cream. The values obtained indicate that these two specimens of taka-diastase have about the same activity towards starch as that used in experiments 46, 47, 48 and 50.

The observation in the experiments with wheat (Tables III and IV) that increasing the proportion of taka-diastase increases the amount of starch found can only be explained by postulating an incomplete hydrolysis with the smaller amount of enzyme involving the persistence of dextrin which would be co-precipitated by the basic lead acetate solution and by its loss would lower the apparent starch content. In the case of the experiments with pure starch however the evidence

is contradictory. In favour of the hypothesis of the persistence of dextrin are the results recorded in Tables XVIII-XXII and Exp. 14 of Table VII, which show that basic lead acetate solution produces a proportionately greater depression of the optical rotation than of the cupric reducing power of a hydrolysed starch solution, and that the reduction in rotation so produced is larger than can be accounted for by the loss of maltose. Against the hypothesis there are two observations. The first is that increasing the proportion of enzyme or lengthening the duration of hydrolysis very often does not increase the apparent starch content, thus in Table V the results of Exps. 1 and 2 are higher than those of Exps. 3 and 4 in which the amount of enzyme is doubled, that of Exp. 1 is roughly the same as that of Exp. 5 in which both the amount of enzyme and time of hydrolysis are doubled and Exp. 6 gave a higher result than did Exp. 5 although the conditions were the same. Table VI shows similar inconsistencies. It is true that in Exp. 42, 1 gram of enzyme gave a higher result than did either Exps. 41 or 43 in which only 0.1 gram of the same enzyme was used, nevertheless Exp. 46 in which also 0.1 gram was used gave almost as high a result as did Exp. 42. Secondly, even in experiments in which no clarifying agent was used (first half of Exp. 14, Table VII, and divisions (a) of Exps. 46-51, Table XIX) the apparent starch content is still as low as 95.12 to 96.90 per cent., whereas it can be shown by a simple calculation<sup>1</sup> that the presence of 5 per cent. of dextrin (of specific rotatory power  $202^\circ$  and no reducing power) would reduce the apparent starch content to 99.44 per cent., whilst 10 per cent. of the same dextrin would give 98.65 for the apparent starch percentage.

The only definite conclusion that the author feels entitled to draw from the whole of the results described is that the taka-diastase method of estimating starch cannot be implicitly trusted—it is necessary to make test experiments on pure starch with every sample of enzyme both before use and during the time it is kept. On the whole the evidence that the persistence of dextrin is the cause of the low values obtained for the

<sup>1</sup> If it be supposed that in an experiment in which 2 grams of vacuum-dried starch were used, 10 per cent. of this was left in solution as dextrin whilst the remaining 1.8 grams were converted 0.8 gram into maltose and 1 gram into dextrose, then the final solution (500 c.cm.) will contain 0.2 gram of dextrin, 1.111 gram of dextrose and 0.844 gram of maltose. These three substances in the concentrations given would impart to the solution optical rotations of  $0^\circ.323$ ,  $0^\circ.468$  and  $0^\circ.929$  respectively—totalling  $1^\circ.721$ . Twenty-five c.cm. of the solution will reduce 0.2008 gram of copper oxide, 0.1431 gram due to the dextrose and 0.0577 gram due to the maltose. Then by using these two values ( $1^\circ.721$  and 0.2008) in the ordinary starch calculation (*Journ. Agric. Sci.* 1914, 6, 160-61) the amount of starch found as being originally present is 1.9730 or 98.65 per cent. of the weight taken.

apparent starch content seems to have greater weight than that against this hypothesis but it must be admitted that the case is not clear.

The author's grateful thanks are due to Mr C. Revis, Mr H. F. E. Hulton and Messrs Parke, Davis and Co. for the supply of several samples of taka-diastrase, also to Prof. H. E. Armstrong, F.R.S. and Mr W. A. Davis for the great interest they have taken in the work and for much helpful criticism.

NOTE BY HENRY E. ARMSTRONG.

During many years past it has been my hope that a method would be evolved by which starch could be determined not only with accuracy but also with comparative ease: the need and value of such a method must be obvious to all. I therefore welcomed the important advance made by Mr W. A. Davis in his use of Taka diastase: it seemed that the problem was solved. Apparently this is not the case: whatever the reason of Mr Horton's inability to obtain consistent results, he has rendered service by showing that even in hands so careful and skilful as are his the method is subject to drawbacks. The precise reason of his failure is not clear. Personally, I am greatly disappointed, as I have so long desired to secure complete analyses of the Rothamsted cereals—especially of the Wheats—in order that the effect of varying conditions of growth may be properly evaluated. It is scarcely creditable to us as chemists that our power of dealing with such analytical problems is still so limited.

Obviously the use of a mixture of enzymes, uncertain in composition, produced by a method which is in no way open to control, is not to be advocated: the first step would seem to be to study the organisms concerned so that control may be secured over the production of the necessary series of agents in due proportions. The problem can be solved only by properly organised team work.

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## NORMAL GROWTH IN ANIMALS.

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(With Seven Figures in Text.)

THE rate of growth of the animal is a matter of considerable importance in the economics of meat production, but the subject does not appear to have been studied with the close scientific attention it deserves. The literature was reviewed in a recent paper by Hammond<sup>1</sup> and need not be further referred to as it is scanty and uninteresting.

There appears to be some confusion in regard to the meaning of the term "rate of growth." It is often said, for example, that pigs grow faster than cattle; but if a pig and a calf of the same age, or of the same live weight, be kept for a year the latter will be the bigger at the end of that time. It may be said that pigs grow faster in proportion to their size but the statement is ambiguous. Minot<sup>2</sup> divided the period of growth into stages and calculated the increase during each interval as percentage of the weight at the beginning of that stage. By this purely arbitrary method he obtained data for diagrams which are very striking by reason of the exaggeration of the ordinates but are of little use for any scientific purpose. The rate of growth is the increase in size per unit of time, not the ratio of that increase to the original, maximum or any other size.

The precise signification even of the term "growth" itself is dubious. In the case of vertebrate animals it implies essentially extension of the frame. This can only be determined by measurement of dimensions. It is not easy to obtain such measurements accurately in the case of living animals, especially when young, and, owing to the variation in shape, it is difficult to compare them. Even to a casual observer it is obvious that a pig and a sheep are not geometrically similar figures. The difference in shape of a cow and a newly born calf is equally striking. In fully grown cattle the ratio of girth to length (the horizontal distance from the point of the shoulder to the pin bone) varies<sup>3</sup> normally from 1·38 to 1·53. For these reasons, therefore, it is usual to estimate alteration in size, or differ-

<sup>1</sup> *Journ. Agric. Sci.* 1920, 10, p. 3.

<sup>2</sup> *Age, Growth and Death*, Murray, London, 1908.

<sup>3</sup> The Author, British Association, 1914.

ence in size, by the alteration or difference in live weight. This datum, however, depends not only upon the size but also upon the condition (fatness) of the animal, and unless one of these can be discounted the other cannot be determined in this way. A beast may increase from 100 lb. to 1000 lb. without change of condition and subsequently gain an additional 300 lb. or more by fattening. The latter should not be reckoned as growth. A similar animal, if fattened while still young, may increase without change of condition to the same final weight and, in the absence of precise definition of terms, the whole of this increase might be reckoned as growth. The only difference between the two cases, however, is that in the former growth and fattening take place successively whereas in the latter they occur simultaneously. The difficulty cannot be avoided by arbitrarily defining growth as the increase in weight in "store condition." Not only is this term an indefinite one but, even if a precise meaning, e.g. 15 per cent. of body fat, be ascribed to it, "store condition" is, after all, a stage or degree of fatness. The same is true of "very lean" condition and every other except zero. Logically, therefore, growth should be measured by the increase in the fat-free live weight. The latter is easily calculated if the percentage of body fat be known and the author<sup>1</sup> has shown that it may be inferred approximately from the condition as estimated by practical men.

In vertebrate animals the rate of growth may be influenced by a variety of conditions such as food, health, sexual development, fatness, climate, housing, etc. But beyond question the most potent factor is the size of the progenitors. This, it may be assumed, is, normally, the maximum size to which the growing animal can finally attain. It is mainly this condition which forms the subject of the present investigation.

The data relating to the growth of sheep were collected by Mr J. Hammond in conjunction with the late Major P. G. Bailey and Mr K. J. J. Mackenzie at the University Farm, Cambridge, and kindly placed by them at the author's disposal. As they have not hitherto been published they are given in full in the table appended to this article. Graphs were plotted from the same as a preliminary step in the investigation.

In view of the difficulty of deducing formulæ corresponding to given curves it seemed advisable to enquire, in the first place, whether the so-called "surface law" had any bearing on the problem. Basal katabolism, capacity for locomotion and other physiological phenomena are governed by that principle because they depend on the area of cross section; and, like the surface area, this varies as the  $\frac{2}{3}$ rd power of the

<sup>1</sup> *Journ. Agric. Sci.* 1919, 9, p. 11.

volume or of the weight in fat-free condition. If the size of the progeny at birth depends upon the capacity of the uterus it should be subject to the same rule; and such, in fact, appears to be the case. The weight of a calf at birth is, roughly, about  $\frac{1}{20}$ th of the weight of the cow but the weight of the lamb is about  $\frac{1}{10}$ th of that of the ewe. The actual weight of calves varies normally from about 50-100 lb., and that of lambs from 10-20 lb. In other words, while the cow is, in round numbers, ten times the size of the ewe, the calf is only about five times the size of the lamb. This is approximately in the proportion of the  $\frac{2}{3}$ rd power of the weight.

$$\frac{1000}{100} = 10; \quad \frac{1000^{\frac{2}{3}}}{100^{\frac{2}{3}}} = \frac{100}{21.5} = 5 \text{ (nearly).}$$

The farmers' rather crude estimate of the rate of growth exhibits a similar relationship. In the earlier stages of growth cattle increase at an average rate of about 2 lb. per day, and sheep at about 0.4 lb. But the rate of growth diminishes as the animals increase in size and it was thought possible that this acceleration at any stage might be proportional to the weight at that stage. This tentative hypothesis is expressed by the formula:

$$\frac{dm}{dt} = am^{\frac{2}{3}} - bm,$$

where  $m$  is the live weight at any time  $t$  and  $a$  and  $b$  are constants to be found. The curvature of the graphs appeared to be consistent with the hypothesis; investigation of the data showed that it is tenable and that  $a = 1$ . Consequently

$$b = m^{-\frac{1}{3}} = \frac{dm}{dt} \times \frac{1}{m}.$$

The following table shows the value of  $b$ , calculated in this way, for sheep at various stages of growth.

Table I. *Value of b calculated for sheep at various stages of growth.*

1. Females (single).

Time after birth	SUFFOLK SHEEP			SHROPSHIRE x MERINO SHEEP		
	Live weight	Increase per week	$b$	Live weight	Increase per week	$b$
Weeks	lb.	lb.		lb.	lb.	
10	64.4	4.50	0.180	43.1	2.65	0.224
20	104.9	3.10	0.182	67.4	2.45	0.209
30	131.1	2.75	0.176	83.2	1.15	0.218
40	145.5	1.55	0.179	92.5	1.40	0.202
50	155.0	0.90	0.180	105.8	0.85	0.203
		Mean ...	0.179		Mean ...	0.211

## 2. Males (single).

Time after birth	SUFFOLK SHEEP			SHROPSHIRE × MERINO SHEEP		
	Live weight	Increase per week	$b$	Live weight	Increase per week	$b$
Weeks	lb.	lb.		lb.	lb.	
5	43.2	4.8	0.174	29.6	4.05	0.186
10	69.7	5.8	0.170	51.9	4.30	0.185
15	99.7	5.7	0.185	72.5	5.15	0.169
20	119.7	0.8	0.196	85.9	2.85	0.193
25	144.8	5.9	0.150	99.4	2.25	0.193
		Mean ...	0.175		Mean ...	0.185

For females the value of  $b$  approaches constancy as nearly as could be expected. In the case of the males the results are vitiated to some extent by the effects of castration upon the health of the animals and by the incompleteness of the records; they may, however, be regarded as confirmatory evidence. The calculations were made merely to test the thesis at this point.

When the animal attains its maximum size the rate of growth falls to zero. Therefore, if  $M$  be the maximum value of  $m$ ,  $M^{\frac{2}{3}} - bM = 0$  and  $b = M^{-\frac{1}{3}}$ . If this value be substituted for  $b$  the equation becomes more general and may be stated thus:

$$\frac{dm}{dt} = m^{\frac{2}{3}} - mM^{-\frac{1}{3}} = m(m^{-\frac{1}{3}} - M^{-\frac{1}{3}}),$$

or

$$\frac{dm}{dt} = m \left( \frac{1}{3\sqrt{m}} - \frac{1}{3\sqrt{M}} \right).$$

• Since  $b = M^{-\frac{1}{3}}$  therefore  $M = (1/b)^3$ . Consequently if either  $M$  or  $b$  is known the other can be found. It is a comparatively simple matter to ascertain the weights of a considerable number of fully grown animals of any given breed or type and it will generally be found more convenient and more accurate to determine  $M$  and calculate  $b$ ; but in order to test the formula and show the effect of error in  $b$  the final weight has been calculated from the maximum, minimum and average values of  $b$  as given in Table I. The results are as follows:

	Final weight			
	Calculated from the			Found
	Min.	Max.	Mean	
Suffolk sheep (females, single)	165.9	183.4	174.3	164.1
Shropshire × Merino (females, single)	89.0	121.3	106.4	115.6

The figure "found" is merely the weight at the 59th week—the last recorded. The data show that the animals were still slowly gaining weight but that they had nearly attained their maximum size.

By integration we have

$$m^{\frac{1}{3}} = M^{\frac{1}{3}} \left\{ 1 - \frac{D}{e^{\frac{1}{3}t} M^{-\frac{1}{3}}} \right\}.$$

When  $t = 0$   $m$  is the weight at birth and

$$D = (1 - m^{\frac{1}{3}}/M^{\frac{1}{3}}),$$

and since  $D$  is a constant it may be found in this way. The value of  $D$  can of course be found at any stage for which corresponding values of  $t$  and  $m$  are known and when tested in this way it is found to increase progressively. It appears, therefore, that either the original hypothesis  $dm/dt = am^{\frac{2}{3}} - bm$  does not express the whole truth or the coefficient  $a$  is not exact. More numerous observations would give a closer approximation but are not available. The necessary correction can, however, be effected by the introduction of an empirical factor  $(\kappa t^2 + 1)$ . In the case of sheep the constant  $\kappa$  is of the order of  $1/5000$ . The actual values found in the several cases examined were as follows:

	Females		Males		Mean
	Single	Twins	Single	Twins	
Suffolk sheep	·0001	·0001	·000145	·0001	·000173
Shropshire x Merino	·0006	·00028	·00034	·00006	

The complete formula for sheep therefore is:

$$m^{\frac{1}{3}} = M^{\frac{1}{3}} \left\{ 1 - \frac{\left(1 - \frac{m_0^{\frac{1}{3}}}{M^{\frac{1}{3}}}\right) (1 + \cdot00017t^2)}{e^{\frac{1}{3}t} M^{-\frac{1}{3}}} \right\},$$

where  $M$  is the maximum or final weight  $m_0$  is the weight at birth and  $m$  is the weight (in same condition) at any time  $t$ .

On reference to the following table and graphs (Fig. 1) it will be seen that the calculated results agree with the observed data as closely as could be expected. The more important causes to which the differences are attributable may be grouped under three heads, viz.: (1) uncertainties in the data, (2) individuality and (3) the health of the animals.

The data bear internal evidence of a high degree of reliability, but the number of animals under observation—a dozen or twenty in each group—was not large enough entirely to disguise fluctuations due to causes not under consideration. The weight at birth was recorded only in two of the groups and the maximum or final weight not in any. There is every reason to believe that the numbers selected to fill these blanks are very near to the truth, but any errors in these essential data are, of course, reflected throughout the whole series of calculated results.

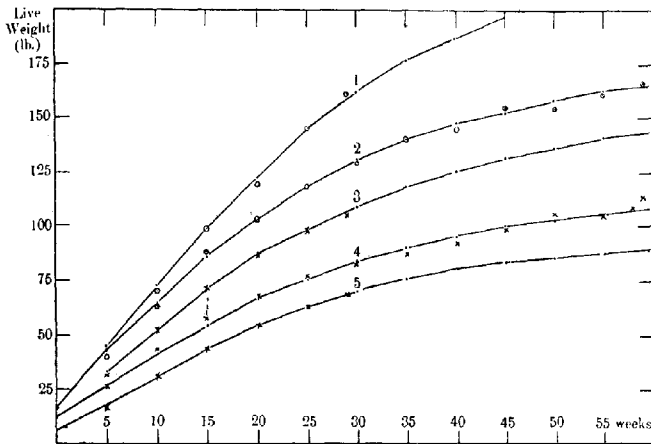


Fig. 1. Growth of sheep.

Calculated live weights on successive dates:

- Curve 1. Suffolk sheep (males, single).  
 " 2. " " (females, single).  
 " 3. Shropshire x Merino Sheep (males, single).  
 " 4. " " (females, single).  
 " 5. " " (females, twins).

The weights actually observed are indicated by  $\odot$  for the Suffolk and by  $\times$  for the Shropshire x Merino sheep.

Table II. *Live weight of sheep, calculated and observed.*

## 1. Females (single).

Time Weeks	SUFFOLK SHEEP			SHROPSHIRE x MERINO SHEEP		
	Calculated lb.	Found lb.	Difference lb.	Calculated lb.	Found lb.	Difference lb.
1	25.9	21.6	+4.3	13.8	17.5	-3.7
5	43.2	39.3	+3.9	25.3	26.5	-1.2
10	65.5	64.4	+1.1	41.8	43.1	-1.3
15	86.3	87.7	-1.4	55.5	58.6	-3.1
20	104.2	104.9	-0.7	67.1	67.4	-0.3
25	119.0	118.8	+0.2	76.5	76.9	-0.4
30	130.9	131.1	-0.2	84.2	83.2	+1.0
35	140.4	140.3	+0.1	90.5	87.3	+3.2
40	147.9	145.5	+2.4	95.9	92.5	+3.4
45	153.7	155.6	-1.9	100.3	98.9	+1.4
50	158.3	155.0	+3.3	104.0	105.8	+1.8
55	161.9	159.8	+2.1	107.0	106.1	-0.9
59	164.4	164.1	+0.3	110.0	115.6	-5.6

Formula:

$$m^{\frac{1}{2}} = 174.33 \left\{ 1 - \frac{0.5(1 + 0.001t^2)}{e^{0.0596t}} \right\}$$

Formula:

$$m^{\frac{1}{2}} = 120.3 \left\{ 1 - \frac{0.55(1 + 0.006t^2)}{e^{0.0757t}} \right\}$$

The term individuality as commonly used is practically meaningless. Strictly it refers to the sum of the variations which cannot be ascribed to known causes. This is a measurable quantity and is expressed by the probable error of the average. It includes fluctuations resulting from minor variations in health and alterations in fatness too slight to be detected by the ordinary, crude tests of "condition."

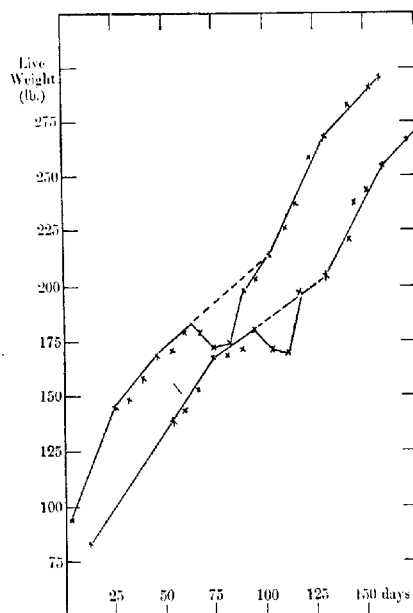


Fig. 2. Effect of castration upon the growth of calves. The observed live weights are shown by x. The broken line indicates the probable normal course of the curve at that stage.

The effects of recognisable ailments have been eliminated by cutting out the individuals affected, but those due to weaning and castration could not be dealt with in that way. The immediate effect of castration is, of course, more readily discerned in the records of individuals. It is clearly illustrated in the graphs (Fig. 2) which represent the earlier stages of growth of two calves. The well marked depression noticeable about the middle of each curve synchronises with the time of castration. The shock of this operation does not appear to have produced any

permanent deflection of the curve of growth. In both cases the curve resumes what appears to be its normal course at that stage within a month. Incidentally these data illustrate the futility of attempting to draw general conclusions from the records of individuals or even small numbers of animals.

These considerations tend to confirm the view that the formula affords a reliable means of estimating the probable weight of sheep at any stage of growth from birth to maximum provided no change of condition occurs. The differences between the observed and calculated data are not such as to occasion any serious misgivings regarding its general accuracy. It remains to be seen whether this formula is applicable to other species.

Rabbits and chickens were selected for investigation because some data were available. It was to be expected that in neither case would the curves of growth follow exactly the same course as those of sheep and that the formula would have to be modified accordingly. The following give curves (Fig. 3) which agree fairly with the observed data:

$$\begin{aligned}\text{Rabbits: } m^{\frac{1}{3}} - 2650^{\frac{1}{3}} \left\{ 1 - \frac{.7363 (1 + .00015t^2)}{e^{.02408t}} \right\}, \\ \text{Chickens: } m^{\frac{1}{3}} - 2200^{\frac{1}{3}} \left\{ 1 - \frac{.74 (1 + .01t^{1.22})}{e^{.02564t}} \right\}.\end{aligned}$$

On reference to the diagram it will be seen that the approximation is closer in the case of the chickens than in that of the rabbits. This is attributable merely to the fact that the observed data were more reliable; the number of animals in the group was larger and the fluctuation in the weights not so wide.

The striking difference in the form of the curves is probably connected with the means of nourishment of the young and illustrates the advantage which mammals possess over other species. At the start the ratio of the live weights was  $49/39 = 1.25$ , but after 20 days it was  $368/126 = 2.92$ ; afterwards it diminished again and at the end the ratio was only  $2650/2200 = 1.2$ . In other words the initial difference in the live weights was only 10 grams; after three weeks the rabbits were nearly three times the size of the chickens but when fully grown they were only 20 per cent. bigger.

In both cases the curves of growth ascend more steeply than those of sheep. The periods of growth are roughly co-extensive—about a year in each case—but in that time rabbits and chickens increase in size more than 50-fold whereas sheep increase only about 10-fold. Apart



from this there is no great difference between the rabbits and sheep. It was shown (Fig. 1) that twins (sheep) are smaller than singles but the curve of growth is much the same. Apparently even the larger number in a litter of rabbits has not much influence upon its shape.

It appears, therefore, that the growth of rabbits and chickens, as well as of sheep, may be represented by the general formula

$$m^{\frac{1}{2}} = M^{\frac{1}{2}} \left\{ 1 - \frac{\left( 1 - \frac{m_0^{\frac{1}{2}}}{M^{\frac{1}{2}}} \right) (\kappa t^n + 1)}{e^{\kappa t} \cdot M^{-\frac{1}{2}}} \right\}.$$

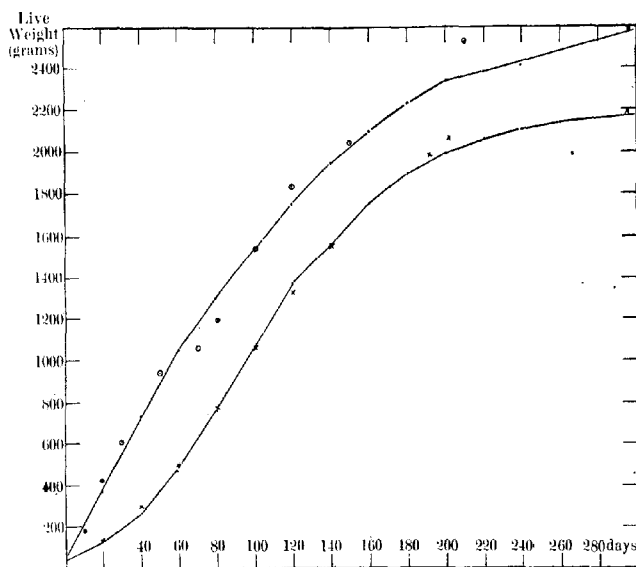


Fig. 3. Growth of rabbits and chickens (females). Calculated live weights on successive dates. The weights actually observed are indicated by © for rabbits and by x for chickens.

The constants  $\kappa$  and  $n$  are not the same for all types and are not easily determined with certainty. An attempt to reason the matter out fundamentally met with no very gratifying success but it helps to define the problem more clearly and as some of the results are not without practical importance it seems desirable briefly to record them.

Growth takes place by processes of cell division. The simplest cases are those of bacteria and other unicellular organisms. In certain species,

under favourable conditions, fission occurs in about 20 minutes<sup>1</sup>. If growth were not restricted by weakening of the nutritive medium, the inhibitory effects of the products of chemical changes induced, or, eventually, by overcrowding of the organisms, the number of cells after a given time would be  $N = n \cdot 2^{\frac{t}{l}}$ , where  $t$  is the time,  $l$  the length of the interval required for cell division and  $n$  the number originally present. This has been verified experimentally<sup>2</sup>. Ordinarily, however, owing to the action of the counteracting agencies referred to, the rate of growth

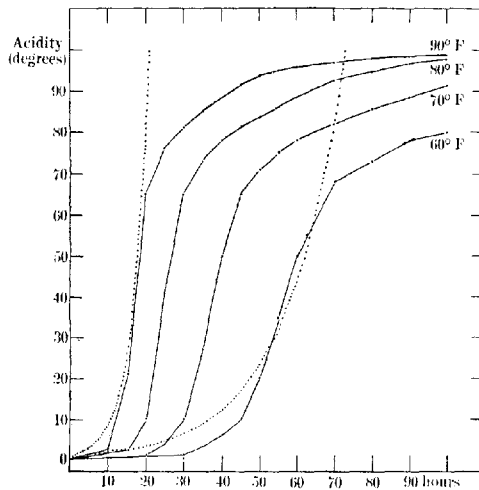


Fig. 4. Souring of milk—degrees of acidity after various intervals of time. The dotted lines show the theoretical maximum at 90° F. and 60° F. respectively, i.e. the acidity which would be produced at any time if there were nothing to inhibit the action of the organisms.

(the number of cells produced per unit of time) increases to a maximum, then gradually diminishes to zero and finally becomes negative in character, i.e. the organisms die out.

The acidity of milk, which results from the growth of certain micro-organisms, increases with and probably in proportion to the number of cells produced but it is not affected by the subsequent diminution of the same. In that respect it resembles the growth of animals. It is therefore a promising line of investigation.

<sup>1</sup> Lane-Clayton, *Journal of Hygiene*, 1909, 9.

<sup>2</sup> Slator, *Trans. Chem. Soc.* 1916, cix. 2.

The graphs (Fig. 4) were plotted from data given by Richmond<sup>1</sup>. The dotted lines correspond to  $N = 23^{\frac{t}{2}}$  and  $N = 21^{\frac{t}{1}}$  respectively. It will be seen that they approximate closely to the curves of acidity at the temperatures indicated up to the point at which milk begins to curdle. Apparently the acid produced does not affect the organisms directly but inhibits their action merely as a result of its effect upon the medium. This view is confirmed by investigations with other species. The difference between the observed and the calculated results at the commencement of the curves is a manifestation of the well-known "bacterial lag." Allowing for this phenomenon, a certain resemblance may be discerned between the curve of acidity at 60° F. and the curve of growth of chickens (Fig. 3). But if any connection exists between the growth of micro-organisms and that of vertebrate animals it is not likely to be revealed by direct comparison. The parallelism, if any, is to be looked for rather in the rate of growth. The rate of increase of acidity of milk, deduced from the data mentioned, is as follows:

Table III.  
*Rate of Increase of Acidity of Milk (degrees).*

Time (hours)	60° F.	70° F.	80° F.	90° F.
0- 10	0.0	0.1	0.2	2.5
10- 20	0.2	1.0	10.0	62.5
20- 30	0.9	9.1	54.8	16.0
30- 40	4.9	39.8	13.0	7.5
40- 50	14.0	21.0	5.5	5.5
50- 60	30.0	7.0	5.0	2.0
60- 70	18.0	4.0	4.5	1.0
70- 80	5.0	3.5	2.0	1.0
80- 90	5.0	3.0	2.0	0.5
90-100	2.0	3.0	1.0	0.5
Total ...	80.0	91.5	98.0	99.0

The distribution of the numbers in each of these series is of the kind produced by binomial expansion. When plotted out, the curves (Fig. 5) resemble ordinary probability curves more or less closely if allowance be made for the bacterial lag. The essential difference in the phenomena is merely one of velocity. It might be supposed therefore that they would all conform to one or other of the various expressions which have been devised to satisfy curves of that kind, for example

$$\frac{dn}{dt} = N(a + b)^{\frac{t}{l}},$$

where  $a$  and  $b$  are constants,  $N$  is the maximum acidity attainable at

<sup>1</sup> *Dairy Chemistry*, 2nd ed. p. 179, Griffin, London, 1914.

the given temperature,  $n$  is the acidity at any time  $t$  and  $l$  is the length of the intervals into which the whole period is divided.

Unfortunately the data in question are inadequate for investigation on these lines. It would be necessary to have more numerous observations with, of course, shorter intervals between them. This would modify the curves to some extent but it would perhaps make it easier to fit them. In any case the curves would be modified in the same sense and the relation between them would be much the same.

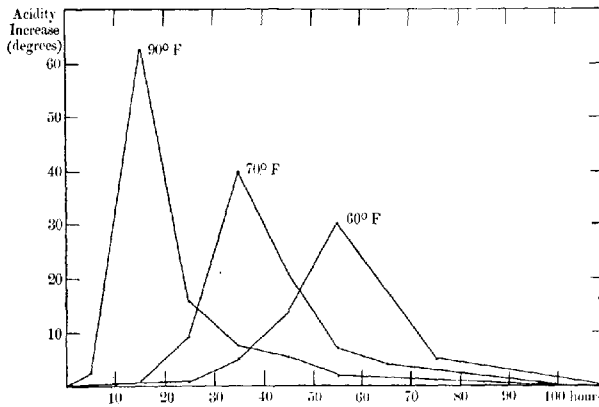


Fig. 5. Souring of milk. Increase of acidity (degrees) in each period of 10 hours, at 60° F., 70° F., and 90° F.

The question is not one of surpassing importance. From the present standpoint it is of interest chiefly in view of the fact that curves representing the rate of growth of rabbits and chickens (Fig. 6) belong, apparently, to corresponding types and bear a similar relation to one another. This is *prima facie* evidence that the difference between the phenomena of growth in rabbits and chickens also is mainly, if not entirely, a question of velocity and that the growth of these animals and of bacteria is governed by the same fundamental law.

It will be seen that the rate of growth attains its maximum, in the case of rabbits, about 30 days and, in the case of chickens, about 90 or 100 days after birth. Sheep apparently attain their maximum rate of growth when only 1 or 2 weeks old, but the point is not very sharply defined as growth continues, with more or less uniformity, almost at the maximum rate, until the animals are from 12 to 15 weeks old.

In practical meat production the largest profit, i.e. the highest ratio of human food (cal.) produced to animal food (cal.) consumed will be obtained if the animals can be brought to the desired condition of fatness precisely at the time they attain their maximum rate of growth. If the two events cannot be made to synchronise, the longer the interval between them the lower will be the ratio referred to.

The curves of growth of rats and guinea pigs are, as might be expected, similar to those of rabbits. But the law which governs these cases is not of universal application. It is well known, for example, that the

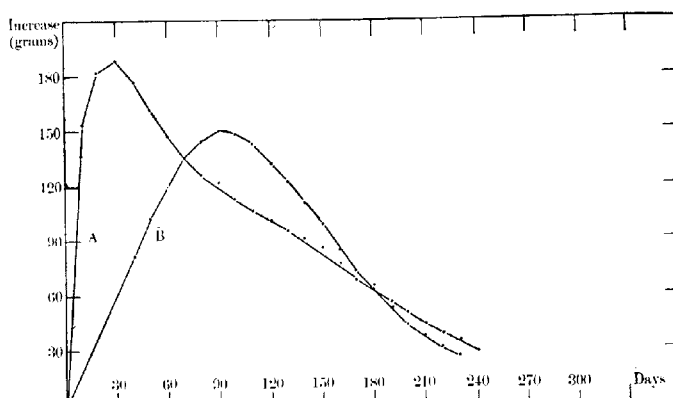


Fig. 6. Growth of rabbits (A) and chickens (B). Increase (grams) per day.

growth of children does not take place with the regularity implied by the smooth curves shown in Fig. 1 and Fig. 3. When the rate of growth from year to year is plotted out the curve is seen to be multimodal. The minor fluctuations are probably without significance from the present standpoint and may be smoothed away; but two well defined modes with a deep sinus between them remain and cannot be ignored. The first occurs probably soon after birth—certainly before the age of one year. After that the rate of growth declines at first sharply and then more slowly until about the fifth year when it begins to increase again. Prior to the tenth year there is no significant difference in the rate of growth of the two sexes, and for clearness, it is represented in the diagram (Fig. 7) by a single line for both. Beyond that point the curves diverge and are shown separately.

The second mode is due to acceleration in the rate of growth. This occurs, in the case of girls, about the age of 12 years and attains its

maximum about a year later. In the case of boys the acceleration is even more striking. It does not begin until the thirteenth year but it continues longer and the maximum, which is reached at the age of 16 years, is much higher than in the case of girls. The fact that in both sexes the acceleration in the rate of growth synchronises with the approach of sexual maturity suggests that these phenomena stand to each other in the relation of cause and effect but there is nothing to show which is which. If such relation does exist one would expect it

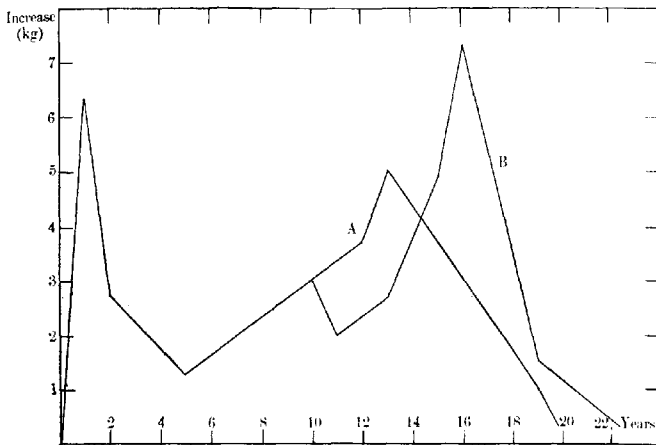


Fig. 7. Growth of girls (A) and boys (B). Increase kg. per annum. Prior to the 10th year the rate of growth of both sexes is represented by the same line.

to occur also in other vertebrates, particularly in mammals, but the cases previously examined afford no evidence of anything of the kind. The only hypothesis the author can suggest to account for this is that in these cases growth is so rapid that it is practically complete before sexual development begins. It is, of course, possible that the acceleration of growth and sexual development are merely correlated effects of a common cause.

In regard to the growth of cattle the only data the author has been able to obtain are those from which the graphs (Fig. 2) were plotted and the weight of fat beasts at shows. These are quite inadequate for investigation on the lines described. They are, however, sufficient to show that the simple formula given for sheep, etc., is not applicable to cattle. The evidence points to an acceleration in the rate of growth between

the first and second year, i.e. to a bimodal curve similar, perhaps, to that shown in Fig. 7.

With such conjectural data it is, of course, impossible to form any opinion regarding the time at which cattle should be slaughtered for beef. It is tolerably certain, however, that it will be found advantageous to kill at one of the modal periods—if indeed there be more than one—or as soon thereafter as the animal can be brought to the required condition of fatness. In this connection it may be recalled that the committee appointed to consider possible economies in food production during the war recommended<sup>1</sup> that cattle should be slaughtered at the age of 17 months. The reasons for selecting this particular age rather than any other, for example 16 or 18 months, were not given in detail and the advice was questioned in several quarters. If, however, the inference that a second mode occurs in the rate of growth curve, at or about the age mentioned, should prove to be well founded the recommendation would appear to be justified.

It was precisely to determine questions of this kind that the present investigation was undertaken. The discovery of a marked difference between the curves of growth of sheep and those of cattle has rendered the attempt abortive. The enquiry having proved so much more involved than was anticipated it would be impossible in any case to pursue it in this article but it is much to be regretted that the necessary data in regard to cattle are not obtainable.

The author desires to acknowledge his indebtedness to Mr J. P. Clatworthy for assistance and advice in connection with the mathematical work in this paper.

<sup>1</sup> *Jour. Bd. of Agric.* vol. XXIII. pp. 986 and 1051.

## APPENDIX.

## GROWTH OF SHEEP.

*Live weights on successive dates.*

## Females (single)

SUFFOLK SHEEP						SHROPSHIRE X MERINO SHEEP					
Age (wks)	Observa- tions	Av. live wt (lbs.)	Age (wks)	Observa- tions	Av. live wt (lbs.)	Age (wks)	Observa- tions	Av. live wt (lbs.)	Age (wks)	Observa- tions	Av. live wt (lbs.)
Birth	2	16.0	30	12	131.1	Birth	—	—	30	23	83.2
1	8	21.6	31	11	134.0	1	2	17.5	31	23	84.4
2	16	23.7	32	11	135.4	2	13	18.7	32	23	84.9
3	16	28.7	33	11	137.0	3	19	20.7	33	23	85.2
4	16	33.8	34	11	138.2	4	21	22.6	34	23	86.5
5	16	39.3	35	11	140.3	5	23	25.5	35	23	87.3
6	16	44.6	36	11	142.2	6	23	30.2	36	23	88.2
7	16	49.1	37	11	143.3	7	23	33.9	37	23	89.0
8	16	54.5	38	11	142.2	8	23	38.0	38	23	89.9
9	16	60.1	39	11	143.9	9	23	41.5	39	23	91.2
10	16	64.4	40	11	145.5	10	23	43.1	40	23	92.5
11	16	61.9	41	11	147.0	11	23	46.8	41	23	94.0
12	16	74.2	42	11	149.4	12	23	49.9	42	23	95.5
13	16	79.3	43	11	151.7	13	23	53.2	43	22	96.6
14	16	83.6	44	11	153.7	14	23	56.7	44	22	97.6
15	15	87.7	45	11	155.6	15	23	58.6	45	22	98.9
16	15	90.7	46	11	155.6	16	23	60.1	46	22	100.2
17	15	94.0	47	11	154.1	17	23	61.8	47	22	101.7
18	15	96.8	48	11	154.3	18	23	64.2	48	22	103.2
19	14	101.7	49	11	154.1	19	23	65.3	49	22	104.5
20	14	104.9	50	11	155.0	20	23	67.4	50	22	105.8
21	14	107.9	51	11	155.9	21	23	70.2	51	22	106.2
22	14	111.3	52	11	157.9	22	23	72.0	52	22	106.6
23	14	113.9	53	11	158.3	23	23	73.4	53	22	106.1
24	13	115.9	54	11	159.5	24	23	75.3	54	22	105.6
25	13	118.8	55	11	159.8	25	23	76.9	55	22	106.1
26	13	121.0	56	11	161.0	26	23	77.9	56	22	106.6
27	13	123.4	57	11	160.8	27	23	79.1	57	22	107.3
28	13	125.3	58	9	162.2	28	23	81.0	58	20	110.0
29	12	128.5	59	9	164.1	29	23	82.1	59	15	115.6



Age (weeks)	Females (Twins)			Males (Single)			Males (Twins)		
	SUFFOLK		SHROPSHIRE × MERINO	SUFFOLK		SHROPSHIRE × MERINO	SUFFOLK		SHROPSHIRE × MERINO
	Observa- tions	Average live weight (lbs.)		Observa- tions	Average live weight (lbs.)		Observa- tions	Average live weight (lbs.)	
Birth	10	11.2	5	14	20.2	3	18	15.2	3
1	15	16.1	5	18	25.9	14	21	19.2	3
2	17	19.3	6	18	31.3	17	21	22.6	9
3	17	23.2	14	18	37.3	17	21	26.5	9
4	18	26.7	14	18	43.2	18	20	31.9	9
5	18	30.5	16	18	46.9	18	20	35.3	9
6	18	34.5	16	18	51.5	18	20	45.1	9
7	18	39.1	16	18	58.5	18	20	50.9	9
8	18	43.5	16	18	64.1	18	20	54.4	9
9	18	48.3	16	18	69.7	18	20	60.7	9
10	18	52.9	16	18	75.7	18	20	66.6	9
11	18	58.1	16	18	82.1	18	20	70.5	9
12	18	62.6	16	16	87.1	18	19	74.4	9
13	18	66.8	16	16	94.4	18	19	79.4	9
14	18	70.7	16	16	99.7	18	18	84.7	9
15	17	74.8	16	12	105.8	18	18	88.7	9
16	17	78.0	16	12	110.5	18	18	90.9	9
17	16	81.5	16	12	117.3	18	15	90.4	9
18	16	82.4	16	11	119.2	18	14	90.9	9
19	16	90.0	16	11	129.8	18	14	93.9	9
20	16	93.1	16	10	135.1	18	11	98.1	9
21	16	96.2	16	9	133.1	18	7	102.6	9
22	10	97.3	16	9	138.9	18	2	117.0	9
23	8	102.7	16	9	144.8	18	2	122.0	9
24	5	105.6	16	9	150.7	18	2	126.5	9
25	3	114.0	16	8	155.5	18	1	141.0	9
26	3	115.6	16	8	160.0	18	—	—	9
27	3	117.3	16	4	164.3	18	—	—	9
28	3	119.6	16	3	—	18	—	—	9
29	3	121.6	16	3	—	18	—	—	9

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## ERRORS IN FEEDING EXPERIMENTS WITH CROSS-BRED PIGS.

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(With Two Figures in Text.)

### INTRODUCTION.

A SERIES of pig-feeding experiments<sup>1</sup> was commenced at the West of Scotland Agricultural College Experiment Station, Kilmarnock, in 1905. In these experiments cross-bred Yorkshire pigs were employed. They were grouped into lots of four, in such a way that each lot had as nearly as possible the same total initial live weight. The pigs in each lot were fed on a different ration, the other experimental conditions being the same for all the groups. The pigs were weighed at fixed intervals and the difference in the total gain between the lots was taken as a measure of the difference in the fattening value of the ration used. Agreement between the results of similar experiments repeated several years in succession was not close. This was due to the normal variation in the rate of fattening of the individual cross-bred pigs used in the experiments which is generally included in and expressed by the probable error of the experiment.

Errors in feeding experiments have been the subject of several papers which have appeared in previous issues of this and other *Journals*. The probable error, calculated by the usual statistical method, was found by different investigators to be as follows:

		Number of animals	Probable error on one animal calculated as a percentage of the live weight increase
Wood and Stratton <sup>2</sup>	...	100 cattle	14
Robinson and Halnan <sup>3</sup>	...	13 pigs	8
Berry <sup>4</sup>	...	76 "	12
Crowther <sup>5</sup>	...	9 "	3.5

<sup>1</sup> "Report on Seven Experiments on the Feeding of Pigs" (*Bull.* 57, Tenth and Eleventh Annual Report) West of Scotland Agric. College, Glasgow.

<sup>2</sup> Wood, T. B. and Stratton, F. J. M., "Interpretation of Experimental Results." *This Journal*, 3, pt. iv, 1910, pp. 417-440.

<sup>3</sup> Robinson, G. W. and Halnan, E. T., "Probable Error in Pig Feeding Trials." *This Journal*, 5, pt. i, 1912, pp. 48-51.

<sup>4</sup> Berry, R. A., "Errors in Pig Feeding Experiments." *Science Progress*, Jan. 1913, No. 27. *Trans. British Assoc.*, Sec. 1-9, 1912.

<sup>5</sup> Crowther, Charles, "Probable Error in Pig Feeding Trials." *This Journal*, 7, pt. ii, 1915, pp. 137-142; see also Mitchell and Grindley, "Element of Uncertainty in the Interpretation of Feeding Experiments," University of Illinois, Agric. Exp. Station, *Bull.* 165, 1913, July.

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According to these figures the probable error on one pig may be as low as 3.5 and as high as 12 per cent. calculated on the average live weight increase of the pigs under experiment. In view of the lack of agreement between these results it was decided to carry out pig-feeding experiments at Kilmarnock in order to obtain data bearing on the normal variation in the rate of fattening of cross-bred pigs, and further to investigate the effects of factors such as the influence of sex, the duration of the experiment, etc. on the probable error.

### PLAN OF EXPERIMENT.

In 1912, 43 and in 1913, 46 cross-bred pigs were placed under experiment. In both years the experiments which were identical in their details were carried out during the summer months, and they were in each case of 16 weeks' duration. It is, therefore, possible to combine the two experiments. The pigs in both years were arranged four to a pen and each pen had four feeding troughs so that individual feeding was, to a certain extent, possible. All the pigs in the different pens were fed alike. The meals, which consisted of equal parts by weight of thirds, bran and maize, were scalded to a porridge with whey and given in a sloppy condition at 6 a.m. and at 5 p.m. At noon whey only was given. The daily allowance of meal at the commencement was 2 lbs. per head. This allowance was increased as the experiment proceeded until at the end of the experiment the pigs were receiving 4 lbs. of meal per head per day. The allowance of whey at first was 3 gallons per day for each animal, and this was increased to 4 gallons after the second month. The quantity of food supplied during any period of the trial was that which was found to be sufficient to allow the animals to feed to repletion. The pigs were weighed separately each week throughout the experiment. The weighing was done on the same day and at the same hour each week.

The initial live weight, the average daily increase, and the sex of each of the 89 pigs are given in Table I, p. 277. The animals are arranged in the table according to the average daily live weight increase of the individual pigs commencing with the slowest fattening pig.

The slowest fattening pig showed an average daily increase in live weight of 1.0 lb. and the quickest of 1.73 lbs., a difference in the rate of fattening between the two of 73 per cent. The average rate of fattening for the 89 pigs was 1.38 lbs. per day.

The four pigs in the slowest fattening pen put on flesh at the rate of 1.25 lbs. per day, whilst the four pigs in the quickest fattening pen

fattened at the rate of 1.43 lbs. per day, a difference in the rate of fattening between the animals in the two extreme pens of 14.5 per cent., calculated on the average rate of fattening of the four pigs in the slowest fattening pen. Theoretically it is possible to group the four slowest and the four quickest fattening of the 89 pigs into pens. If that were done the difference in the average rate of fattening of the pigs in the two pens would be as great as 70 per cent. but the chances against selecting in one pen the four slowest or the four quickest fattening animals are exceedingly high, calculated mathematically they are about 1 to 2,440,000. With care in the selection and grouping of the animals into pens of four, the greatest difference in the average rate of fattening of two pens is very much less than the maximum, being only 14.5 per cent.

Table I.

Initial live weight lbs.	Average daily increase lbs.	Sex	Initial live weight lbs.	Average daily increase lbs.	Sex	Initial live weight lbs.	Average daily increase lbs.	Sex
48	1.00	M	76	1.32	M	60	1.44	M
63	1.04	F	59	1.32	F	70	1.45	M
68	1.13	M	74	1.32	M	76	1.45	F
64	1.17	M	58	1.33	F	92	1.45	M
79	1.18	F	74	1.33	F	82	1.46	F
82	1.18	M	78	1.35	M	69	1.46	F
70	1.19	M	64	1.35	F	75	1.46	M
60	1.20	F	78	1.35	F	63	1.46	M
70	1.21	F	68	1.35	F	82	1.46	F
66	1.23	F	57	1.35	F	72	1.46	F
61	1.24	M	80	1.36	F	74	1.47	M
67	1.25	F	82	1.37	M	64	1.47	M
71	1.25	F	90	1.37	M	63	1.48	M
86	1.25	M	66	1.38	F	76	1.48	F
68	1.26	M	67	1.38	M	59	1.50	M
67	1.26	F	68	1.38	F	78	1.51	M
58	1.26	F	72	1.38	F	90	1.51	M
76	1.27	F	82	1.39	F	72	1.53	F
69	1.28	M	64	1.40	M	80	1.53	M
74	1.28	M	68	1.40	F	94	1.57	M
64	1.28	F	67	1.40	M	68	1.59	F
68	1.28	F	82	1.41	F	74	1.59	F
52	1.28	M	70	1.41	F	74	1.59	M
68	1.28	M	78	1.42	M	85	1.62	M
110	1.29	M	71	1.42	M	88	1.63	F
69	1.29	F	65	1.42	F	58	1.64	F
62	1.30	M	70	1.42	F	60	1.65	M
61	1.30	M	66	1.43	F	95	1.72	M
63	1.31	F	89	1.44	F	112	1.73	M
72	1.31	M	70	1.44	F	—	—	—
			Average live weight			72	1.38	Average

## FREQUENCY CURVES.

In order to show whether the variation in the rate of fattening between individual pigs was a normal one, a frequency curve was plotted from the average daily live weight increase for each pig, Table I, p. 277. Except for a slight divergence at the right-hand end, the curve was found to correspond fairly closely to a mathematical normal frequency curve. It was thought that this irregularity in the curve might be caused either by the selection of pigs of both sexes, or by the fact that the pigs were cross-breds and that insufficient numbers were being dealt with. The male and female pigs were, therefore, tabulated separately, and from the tables frequency curves for each sex were constructed. From these two curves it became quite clear that the inclusion of the female pigs accounted for the irregularity mentioned above.

## SEX AND RATE OF FATTENING.

Of the 89 pigs (Table I, p. 277) 41 are male and 45 female, the former had an average initial live weight of 74 lbs. and showed an average daily increase of 1.39 lbs., whilst for the latter the corresponding figures are 70 lbs. and 1.37 lbs.

On comparing the sex of the pigs which fatten most slowly, and taking the pigs in groups of ten, as they occur in Table I, it is found that in the first three consecutive groups of ten animals, male and female pigs occur in almost equal numbers. Starting at the other end of the table and taking the pigs which fatten most rapidly in groups of ten it is found that in the thirty quickest fattening animals the proportion of male to female pigs is 2 to 1. Of the 29 pigs left which are intermediate in their rate of fattening the proportion of females to males is approximately 2 to 1.

The distribution of the sexes in the above groups seems to us to point to the following conclusions: (1) That it is only the quickest fattening female pigs that are affected by sex. (2) As a result of sexual disturbance the normal rate of fattening of some female pigs is diminished. The female pigs which normally should occur in the quickest fattening group appear in the intermediate group. It also establishes the fact that speyed female pigs are more suitable for feeding experiments than unspeyed pigs.

## PROBABLE ERROR AND BASIS OF CALCULATION.

In the case of cattle and sheep Wood and Stratton used as the basis for the calculation of the probable error the average daily increase per 1000 lbs. live weight. Whilst Robinson and Halnan, in the case of pigs,

calculated the error on the basis of the actual average daily live weight increase. In the present paper both methods have been adopted and the figures obtained are given in Table II:

Table II.

Number of pigs	Average initial live weight lbs.	Per 1000 lbs. live weight		Actual average daily increase of each pig (lbs.)	Percentage probable error
		Average daily increase of each pig (lbs.)	Percentage probable error		
43	76	18.31	8.79	1.37	7.58
46	68	20.52	8.98	1.39	5.88
89	72	19.45	9.67	1.38	6.71

The percentage probable error is greater when calculated on the basis of 1000 lbs. live weight. The effect of the calculation on this basis is to exaggerate out of all proportion the differences between the rate of fattening of individual pigs. Moreover no useful purpose is gained by calculating on a 1000 lbs. standard, a weight which pigs never at any time attain.

The probable error has been calculated separately for the male and female pigs and the result is given in Table III:

Table III.

Number of pigs	Sex	Average initial live weight lbs.	Average difference of each pig from average initial live weight lbs.	Average daily increase of each pig lbs.	Probable error percentage
44	Male	74	10.8	1.39	7.45
45	Female	70	6.1	1.37	5.95

Here the effect of the greater extremes of variation in the initial live weights in the males compared with that of the females is indicated by the higher probable error of the former.

#### PROBABLE ERROR AND INITIAL LIVE WEIGHT.

In the 43 pigs in the 1912 experiment the initial live weight of the pigs varied from 57 to 112 lbs. with an average live weight of 76 lbs. In the 46 pigs in the 1913 experiment the variation in the initial live weight of the pigs was less, namely from 48 to 90 lbs., the average live weight being 72 lbs. The average difference of each pig from the average initial live weight in the former experiment was 10.4 lbs., in the latter 5.9 lbs. for the two combined 8.4. For the male pigs it was 10.8 lbs., and for the female pigs 6.1 lbs.

If the variation in the initial live weight influences the probable error

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then it would be expected that the error on the 43 pigs would be greater than the error on the 46 pigs, and the error on the 46 pigs would be less than that on the 89 pigs. This is found to be the case, see Table II, p. 279. If we apply the same reasoning to the male and female pigs, the probable error of the female pigs should be considerably less than that for the male pigs. This is borne out by Table III, p. 279.

The connection between initial live weight and probable error is further shown in Table IV, below. The pigs are arranged into groups according to their initial live weight.

Table IV.

Number of pigs	Initial live weight lbs.	Average initial live weight lbs.	Average difference of each pig from average initial live weight lbs.	Average daily increase of each pig lbs.	Percentage probable error
7	50 to 59	57	1.7	1.38	6.67
34	60 „ 69	65	2.5	1.34	6.34
28	70 „ 79	74	2.4	1.39	5.30
12	80 „ 89	83	2.2	1.42	6.26
5	90 „ 99	92	1.8	1.52	5.88
56	—	72	8.4	1.38	6.71

Unfortunately the pigs of the respective groups are too few in number to provide a trustworthy comparison. However the error obtained for each of the groups is always less than the error for the total number of pigs, indicating that the closer the pigs are to each other in their initial live weights the less will be the probable error. Column 5, Table IV, indicates that pigs with higher initial live weights show a higher average daily live weight increase. In other words the heavier the pig is to begin with the greater, as a rule, is the daily increase. Hence it follows that great variation in the initial live weight tends to cause a correspondingly wide variation in the live weight increase and consequently increases the probable error. In pig feeding experiments, therefore, pigs with as near as possible the same initial live weight should be taken.

It would seem from the data at our disposal that the error is proportional to the mean difference of each pig from the average initial live weight and to the average daily increase.

For cross-bred pigs of the same initial live weight the probable error on one pig appears to be approximately 4 per cent., and for every difference of 1 lb. from the average initial live weight the correcting factor works out to be 0.33 per cent. For the 89 pigs the average difference of each pig from the average initial live weight is 8.4 lbs. Applying the

above factor the correction would be  $8.4 \times .33 = 2.77$  per cent., then the probable error would be  $2.77 + 4 = 6.77$  per cent.

For the sake of comparison the percentage probable error calculated on this basis and in the usual way is given in the following table:

				Usual method	Estimated
43 pigs	...	...	...	7.58	7.43
46 "	...	...	...	5.88	5.95
89 "	...	...	...	6.71	6.77
44 male pigs	...	...	...	7.45	7.56
45 female pigs	...	...	...	5.95	6.00

The close agreement between the two sets of figures indicates that the assumption, underlying the method of calculation employed above, is sound, in so far, at all events, as it applies to the cross-bred pigs in the present experiment.

#### PROBABLE ERROR AND BREEDING.

There were included in the 46 pigs in the 1913 experiment 6 pigs from the same litter. Their initial live weights were almost identical. The details of these pigs are given in Table V below.

Table V.

Initial live weight of pig	Average daily increase of each pig
lbs.	lbs.
58	1.26
63	1.31
57	1.32
59	1.35
60	1.20
64	1.28
Average ... 60	Average ... 1.29
Probable error percentage ...	2.71

The pigs fattened very evenly and the probable error is only 2.71 per cent. With cross-bred pigs of the same initial live weight but from different parents the probable error is 4 per cent. of the average live weight increase. From this it would seem to follow that pigs from the same parents do not show such a divergence in the rate of fattening as do pigs from different parents of the same breed. American results<sup>1</sup>, especially, show that animals from different breeds differ markedly in their rate of fattening. Each breed of pigs appears to possess a more or less distinct rate of fattening<sup>2</sup>.

<sup>1</sup> Iowa State College. *Bull.* 91.

<sup>2</sup> The average rate of fattening of any breed taken as a whole will depend upon the attention which breeders have paid to developing this characteristic of the breed. There are strains in all breeds of pigs which are capable of fattening rapidly. In some breeds these strains have been neglected, in others developed.



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Results obtained by Crowther<sup>1</sup>, and by experimenters at the Wisconsin and Michigan experiment station, confirm our conclusions. By careful selection of the pigs according to age, breeding, and initial live weight, it would appear to be possible to reduce the probable error to a low figure.

As the rate of fattening of individual pigs appears to be an hereditary characteristic, it would seem to be a feasible proposition for a large breeder of pigs to establish within a comparatively short time a strain of pigs with rapid fattening qualities. In other words to breed pigs for rapid pork production, just in the same way as it is possible to breed cows with a view to improving their milk production. Owing to the comparatively short time it takes the pig to reach maturity, and also because of the large number of pigs in a litter, a quick fattening pig could be bred much more quickly than a heavy milking cow.

### PROBABLE ERROR AND DURATION OF EXPERIMENT.

The length of time which it is necessary to continue a feeding experiment so as to eliminate, as far as possible, the influence of the time factor on the probable error is a point of practical interest. In order to determine this the percentage probable error for the 46 pigs, which were under experiment in 1913 for 16 weeks, has been worked out for different periods. As an example in order to obtain the probable error of a one week experiment the error has been calculated for each of the 16 weeks on the assumption that each week was a complete experiment in itself. The same method was followed in arriving at the error for a two up to a 16 week experiment. The summarised results are given in Table VI, p. 283.

As would be expected, the figures in Table VI show that the probable error is greatest in experiments lasting one week. As the period of experiment is lengthened the error falls rapidly at first, and after the sixth or seventh week the fall is very slight. In so far as the duration of the experiment brings about a reduction in the probable error no advantage is apparently gained by prolonging the experiment beyond eight weeks. The figures are represented graphically in Fig. 1 below.

With short period experiments the variation in the probable error and in the average daily increase is considerable. For example in 16 weekly experiments the highest error was 24·3 per cent. and the lowest was 14·1 per cent., the average being 18·4 per cent. of the average daily increase. The highest average daily live weight increase in the weekly

<sup>1</sup> Crowther, Charles. *Loc. cit.*

series of experiments was 2.12 lbs. and the lowest 1.05, Table VI. By representing the weekly figures in the form of a curve, Fig. 2 below, interesting relationships between the error and the daily increase are apparent.

Table VI.

Duration of experi- ment weeks	Percentage probable error				Daily live weight increase (lbs.)		
	Minimum	Maximum	Difference between maximum and minimum		Minimum	Maximum	Average
			minimum	Average			
1	14.13	24.33	10.2	18.44	1.05	2.12	1.38
2	9.02	14.25	5.23	11.86	1.11	1.81	1.40
3	8.44	11.93	3.49	9.80	1.17	1.73	1.41
4	7.39	10.51	3.12	8.42	1.21	1.68	1.41
5	7.27	8.48	1.21	7.91	1.23	1.60	1.42
6	6.89	7.95	1.06	7.45	1.23	1.53	1.41
7	6.54	7.78	1.24	7.03	1.25	1.57	1.41
8	6.28	7.40	1.12	7.00	1.32	1.54	1.41
9	6.05	7.35	1.30	6.79	1.31	1.51	1.42
10	6.17	7.10	0.93	6.66	1.29	1.49	1.41
11	6.03	6.70	0.67	6.53	1.31	1.46	1.41
12	6.14	6.79	0.65	6.41	1.38	1.46	1.42
13	5.97	6.80	0.83	6.23	1.38	1.45	1.42
14	5.80	6.32	0.52	6.04	1.41	1.43	1.42
15	5.98	6.26	0.28	6.12	1.40	1.40	1.40
16	5.88	—	—	5.88	—	—	1.39

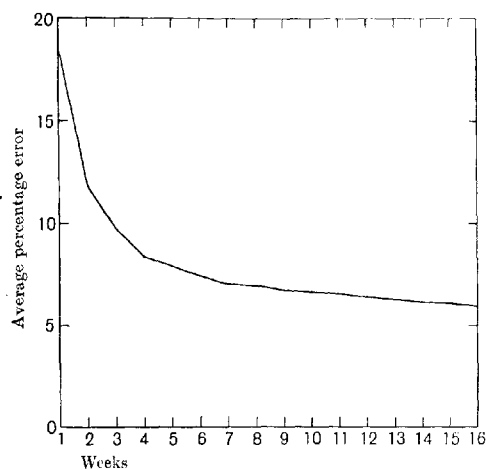


Fig. 1.

The fluctuation in the percentage probable error from week to week is almost in inverse ratio to the average weekly increase. A period of

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quick fattening seems to be invariably followed by a period of depression. Evidently the pig is not an animal which fattens at a uniform rate. If daily weighings were taken, greater variations still would no doubt be found.

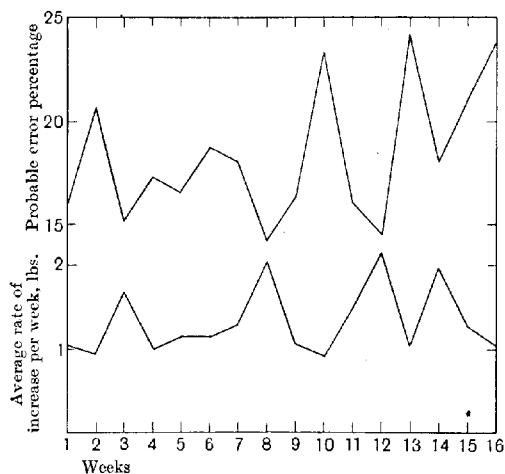


Fig. 2.

### PROBABLE ERROR AND STAGE OF FATTENING.

In all the above experiments lasting different periods it was noticed that as the fattening stage advanced there was no diminution in the probable error. For example in the one week periods the 13th one week period gave the highest and the 16th the second highest probable error, i.e. the greatest variation in the rate of fattening and the largest error occurs in the later stages of fattening.

### MAXIMUM AND MINIMUM ERRORS.

The maximum and minimum errors possible in experiments using varying numbers of cross-bred pigs have been calculated from the 89 pigs given in Table I. The maximum error was calculated by selecting the results of an equal number of pigs showing the highest and lowest daily rate of increase respectively. As a rule this grouping would give the greatest variation in the daily increase, and, therefore, the greatest probable error. The minimum error was calculated on equal numbers of pigs taken from either side of the average daily increase for the whole 89. The result is given in Table VII below.

Table VII.

Number of pigs	Maximum		Minimum	
	Average daily live weight increase lbs.	Percentage probable error	Average daily live weight increase lbs.	Percentage probable error
4	1.37	19.9	1.39	—
8	1.38	15.8	1.39	0.48
16	1.39	13.4	1.39	0.83
24	1.39	11.5	1.39	1.45
32	1.39	10.4	1.39	1.87
40	1.38	9.5	1.39	2.30
48	1.38	8.9	1.39	2.68

The odds against such chance combinations are great and can be worked out by the mathematical formula of chances, yet it must be remembered that the probable error, in pig-feeding experiments where only four pigs are selected for each group, may be as high as the maximum error for that number namely 19.9 per cent.

It was explained, p. 276, that the pigs were arranged in such a way that the total initial live weight of the four pigs in each pen was as nearly as possible the same and the sexes were equally balanced in each group. The error on each pen has been worked out and the result is given in Table VIII below.

Table VIII.

Percentage probable error on 18 pens of four pigs each, in ascending order

2.30	4.20	5.43	7.17	10.76
3.03	4.76	5.50	8.53	12.08
3.13	5.00	5.78	9.67	Average 6.21
3.55	5.07	5.87	9.98	

The lowest percentage error was 2.3 and the highest 12.08. This shows that the variation in the daily increase in weight of four pigs carefully selected and grouped, is such that a minimum error of 12 per cent. must be allowed in all experiments where careful grouping is practised.

Assuming for cross-bred pigs the probable error on one pig to be 7 per cent. of the average live weight increase and that the least odds which can be taken to give a result amounting to a certainty is a 30 to 1 chance, the number of animals required to establish a 5, 10 or 20 per cent. difference in the rate of fattening between two rations has been calculated, and the figures are given below.

Percentage difference in live weight increase between the rations tested	Number of pigs required for each group	
	If difference is uncertain	If difference in one direction is certain
5	40	29
10	10	8
20	3	2

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In most pig feeding experiments with different rations or with different methods of feeding there would not be a greater difference in the rate of fattening than from 5 to 10 per cent.

### SUMMARY.

The probable error on one pig calculated on the average daily live weight increase was found to be for cross-bred pigs about 7 per cent.; for pure-bred pigs from the same litter and each of similar live weight about 3 per cent.; for cross-bred pigs each of similar live weight but from different parents about 4 per cent.

The error is greatest in experiments of short duration and it decreases as the period of the experiment is lengthened. The decrease is slight if the experiment is prolonged beyond seven weeks.

The errors on 18 separate pens containing four pigs to each pen (the total initial live weight of each pen being similar) varied from 2.3 per cent. to 12.08 per cent., the average being 6.21 per cent.

The percentage probable error is relatively low when the pigs are fattening rapidly and high when the pigs are fattening slowly. The average daily increase of the quicker fattening female pigs when unspeyed appears to be depressed as a result of periodic sexual disturbances.

The stage of fattening does not appear to influence the probable error. Animals in the later stages of fattening show a greater range in their rate of fattening than animals in the early stages, see Fig. 2, p. 284.

Weekly weighing of individual pigs is recommended as it gives an indication of the health of each pig. It also enables each experimenter to discriminate between differences in the rate of fattening due to individuality and differences due to ill health, etc.

The rate of fattening of pigs is very variable, periods of rapid fattening are followed by periods of depression.

*(Received February 4th, 1921.)*

# THE EFFECT OF BASIC SLAG UPON GRASSLAND, AND UPON THE CORN CROPS OBTAINED WHEN THAT GRASSLAND IS PLOUGHED UP<sup>1</sup>.

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IN the year 1904, a grass field at Saxmundham Experiment Station was taken in hand by East Suffolk County Council. At that time the herbage in the field was very poor and consisted chiefly of weeds—largely Wild Carrot.

The soil is a typical chalky boulder clay of a poor type very stiff to work, but capable of producing good crops of wheat and beans when well drained.

Considerable areas of such soil exist in the Eastern Counties, and in the period of agricultural depression of the nineties, much of this land was allowed to seed itself down to rubbishy grass—the stubbles being merely left to clothe themselves with a poor herbage. An analysis of the soil of the adjoining field, which is very similar in character, made at Cambridge, gave the following results:

				% in soil dried at 100°
Organic matter and loss on ignition	...	...	...	5.79
Nitrogen	...	...	...	0.178
Carbonate of lime	...	...	...	2.84
Phosphoric acid soluble in strong hydrochloric acid	...	...	...	0.248
Phosphoric acid soluble in 1 % citric acid	...	...	...	0.0213
Potash sol. in strong hydrochloric acid	...	...	...	0.71
„ „ 1 % citric acid	...	...	...	0.002

Two plots, each  $1\frac{3}{4}$  acres in extent, were fenced off with wire netting, and one of these plots received a dressing of 10 cwt. per acre of high quality basic slag, while the other received no treatment. The slag contained 200 lbs. of phosphoric acid. Both plots were grazed with sheep until 1912, when a second dressing of slag (10 cwt. per acre of high quality) was applied to the “slag” plot.

Every year the sheep on both plots were weighed and the increase in live weight noted.

<sup>1</sup> The results of an experiment at Saxmundham.

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Samples of soil were taken by the writer at the close of the sheep experiment and were analysed by the courtesy of Dr Russell of Rothamsted, with the following result:

### *Nitrogen per cent. in dried soil.*

	Basic slag plot %	Unmanured plot %
Soil as far as the depth of turf	0.22	0.20
Soil from end of turf to 9" deep	0.16	0.13
Soil surface to 9" deep	0.17	0.15
Soil from 9" deep to 18" deep	0.11	0.09

Quite a notable gain in nitrogen resulted from the slag treatment.

Taking the weight of the first 9 inches of soil to be  $2\frac{1}{2}$  million lbs. per acre, we see that the basic slag plot contains 500 lbs. of nitrogen per acre more than the unmanured plot, in the first 9 inches of soil and a similar quantity in the second 9 inches, giving a total gain of 1000 lbs. of nitrogen per acre, a quantity equal to that contained in 100 tons of farmyard manure or in 3 tons of nitrate of soda.

The following table gives the increase in live weight of the sheep:

### *Result per acre.*

Plot 1. No manure.

	1905	1906	1907	1908	1909	1910	1911	1912	1913	1914	1915	Total lbs. live weight
Sheep increase in weight (lbs.)	67	78	43	52	70	134	77	73	94	60	78	826

Plot 2. 10 cwt. basic slag applied 1904 and 1912.

Sheep increase in weight (lbs.)	248	194	100	89	100	154	105	103	185	101	142	1512
------------------------------------	-----	-----	-----	----	-----	-----	-----	-----	-----	-----	-----	------

In 1916 the plots were mown, when the "no-manure" plot gave 20 cwt. and the "slag" plot 25 cwt. of hay per acre. The increase in favour of the basic slag is 686 lbs. of mutton per acre.

	£.	s.	d.
Value at $3\frac{1}{2}d.$ per lb. live weight (pre-war prices) ...	10	14	4
Value of 5 cwt. of extra hay at $4s.$ per cwt. (pre-war price) ...	1	0	0
	11	14	4
Cost of manure ...	2	4	10
Total profit over unmanured plot ...	9	9	6
Average profit per acre per annum (pre-war figures) ...	0	17	2

In 1917, owing to war conditions, it was decided to plough up the plots. On ploughing, it was found that there was a much greater thickness of turf (*i.e.* dark vegetable soil) on the slagged plot than on the

"no-manure" plot. In the spring of 1918 oats were drilled. As in so many cases where poor heavy land was ploughed up and sown with oats that crop proved a failure. From experience gained since then, it appears probable that if the land had been ploughed up in December, 1917, and then drilled with peas, or with peas and spring beans in 1918, a satisfactory crop might have been obtained.

Owing to the failure of the oats, it was necessary to fallow the land in 1918.

During the following winter, the land was pipe-drained and in 1919 a mixture of spring beans and Maple peas—2 bushels of beans and  $\frac{3}{4}$  bushel peas per acre—was drilled.

No manure was applied to this crop, but it proved very successful, growing a very large bulk of straw, and giving quite a reasonable yield of corn.

The following was the crop obtained:

1920. *Beans and Peas.*

	Bushels of corn per acre ( $4\frac{1}{2}$ stones per bushel)	Cwts. of straw per acre
No-manure plot ...	29.7	42
Plot manured with slag	40	62

The crop stood up well, and was cut with the binder although the mixed crop formed a somewhat tangled mass. In the autumn of 1919 both plots were ploughed and drilled with Fenman wheat.

A dressing of 3 cwt. of superphosphate (30 per cent.) per acre was applied to both plots in the autumn and 1 cwt. per acre of sulphate of ammonia in the spring. The crop looked well all summer, there being considerably more bulk of straw on the "slag" plot. The following figures were obtained per acre:

1921. *Wheat.*

	Bushels of wheat per acre	Estimated yield of straw per acre (the straw was not weighed)
No-manure plot ...	29 $\frac{1}{2}$	35 cwt.
Plot manured with slag	38 $\frac{1}{2}$	45 ..

It is regretted that it was found impossible to weigh the straw. The yield of wheat was somewhat disappointing on both plots, considering the great bulk of straw.

The crop on the slagged plot was very heavy and would have been



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laid if a heavy storm had occurred. The increase in nitrogen and the consequent increase in fertility of the soil on the slagged plot as compared with that receiving no manure was undoubtedly largely due to the fact that the slag caused a very vigorous growth of wild white clover, and consequently a greater assimilation of nitrogen from the air. It seems probable that the greater thickness of turf on the slagged plot was also due to the greater growth of wild white clover. The effect of the greater accumulation of fertility on the slagged plot seems likely to last for several years.

As all the produce of the plots, with the exception of the hay in 1916, was consumed on the land, most of the nitrogen in the white clover would be returned to the land in the sheep manure.

A certain quantity would be retained on the bodies of the sheep, and "sold off."

The quantity of nitrogen "sold off" the slagged plot, per acre, in excess of that from the no-manure plot would be that contained in the increased live weight of sheep (686 lbs.).

The live weight may be assumed to have contained 2 per cent. of nitrogen (the fasted live weight of store sheep at Rothamsted contained 2.377 per cent. of nitrogen and of fat sheep 1.976 per cent.), so that the 686 lbs. extra weight would contain about  $13\frac{1}{2}$  lbs. of nitrogen, *i.e.* about the quantity contained in 90 lbs. of nitrate of soda. Owing to the poor-ness of the pasture, it was found necessary to give the sheep a certain amount of cake, if those animals were to thrive at all.

A larger number of animals was usually kept on the manured plot, and that plot in consequence had a larger quantity of cake fed upon it. The sheep received  $\frac{1}{4}$  lb. per head daily of decorticated cotton cake, except in the year 1913, when they received  $\frac{1}{2}$  lb. per head daily from June 30th to August 15th. A record was kept of the total quantity of cake fed on each plot. The quantity fed on the slagged plot, in the entire period, exceeded that fed on the no-manure plot by 524 lbs. per acre.

Decorticated cotton cake contains about 7 per cent. of nitrogen so that the total quantity of nitrogen per acre fed on the basic slag plot in excess of that on the no-manure plot was about 36 lbs.

In certain years it was found that the sheep left a rather unsightly quantity of herbage, grass-stems, etc. These were mown and made into hay.

The total amount of hay removed during the whole period from the basic slag plot *in excess* of that from the no-manure plot was 13 cwt. per acre. This, at 1.4 per cent. of nitrogen in hay would contain about

20 lbs. of nitrogen. We thus see that a kind of nitrogen balance sheet can be constructed.

lbs.	lbs.
Amount of nitrogen fed on the basic slag plot, in excess of that fed on the no-manure plot, in the form of cake ... .. 36	Amount of nitrogen sold off the basic slag plot, in the form of mutton, in excess of that from the no-manure plot per acre 13½
	Amount of nitrogen sold off basic slag plot, in the form of hay, in excess of that from the no- manure plot per acre ... 20
	Balance ... .. 2½
36	36

We thus see that the amount of nitrogen in the extra cake fed on the basic slag plot is just about balanced by the amount of nitrogen removed from that plot, in the form of mutton or hay, in excess of the no-manure plot. It is evident, therefore, that the increased fertility of the slagged plot cannot be due to the cake fed. There can be little doubt that it is due to the accumulation of nitrogen and vegetable matter owing to the very vigorous growth of wild white clover on that plot, and this growth was entirely due to the action of 1 ton per acre of basic slag applied in two dressings of 10 cwts., at an interval of eight years.

It is worthy of note that the gain in nitrogen on the slagged as compared with the unmanured plot is only about 10 per cent. whilst the increase in crop is much larger than this. It appears likely that the nitrogen assimilated and stored up by the white clover, being of more recent origin, would be more easily available to plants than the original nitrogen reserves of the soil.

It is also very probable that percentage gain in nitrogen is only a very imperfect way of expressing the gain in fertility of the soil. No doubt the extra plant residues, left on the slagged plot, have a considerable influence on the mechanical condition of the soil, and on its humus content.

The fate of the phosphates applied to the slag plot has not been traced, and this point requires further investigation. It is well known that the presence of phosphate on a heavy soil causes increased root development, but whether there is any increased root development on the slag plot is a point which also requires further study.

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NOTE ADDED JULY 1921

In the autumn of 1920 both plots were ploughed and again sown with wheat, in order to test the amount of residual fertility in the soil. The autumn was very dry, very little rain falling until December. The result of this was that the seed did not germinate satisfactorily and the plant of wheat was much too thin, especially upon the slagged plot. This inferiority of the slagged plot was apparently partly due to the fact that there was a much thicker turf and consequent accumulation of vegetable matter on that plot than where no manure was applied, and that consequently it was much more difficult to get a thoroughly consolidated seed bed, which, as is well known, is so important for wheat.

In March 1921 the wheat was still much too thin, the slagged plot being much thinner than the unmanured plot.

This throws an interesting light upon the conditions favourable or otherwise to wheat. It is quite evident that the light friable soil texture which results after ploughing in a thick turf, although undoubtedly very favourable for certain crops, may under very dry weather conditions render it difficult, if not impossible, to get a solid enough seed bed for wheat and consequently that crop may prove less successful than if a smaller proportion of vegetable matter and decaying grass roots had been present.

In March, it was quite evident that the wheat was much too thin, large numbers of wireworm were also present, so it was considered best to "fill in" the crop with barley. A little more seed barley was drilled where the wheat was thinnest.

In spite of an extremely small rainfall, the barley succeeded fairly well, and writing in July, the mixed crop of barley and a little wheat on the slagged plot looks decidedly better than that on the unmanured plot, but the proportion of wheat present on the latter is greater.

(Received March 23rd, 1921.)

# THE DETERMINATION OF CLAY IN HEAVY SOILS.

By A. F. JOSEPH AND F. J. MARTIN.

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## 1. METHODS OF ANALYSIS.

THE long period of activity shown by investigators in this subject seems to have come to an end nine or ten years ago; from most soil laboratories, the more elaborate elutriation methods have disappeared and only two of the innumerable modifications of clay determination have survived, one as practised in England and the other in America. Both are sedimentation (as opposed to elutriation) methods and both use ammonia for deflocculating the clay. The principal points of difference are that, in America, the time of sedimentation is usually reduced by the use of the centrifuge, and in England, separation of the soil into coarse and fine particles is preceded by treatment with dilute acid in order to remove calcium carbonate, the presence of which is sometimes considered to interfere with deflocculation by ammonia. Workers, who have to deal with very heavy soils containing 50-70 per cent. of clay, find that both these methods leave much to be desired, particularly in respect to the time required for complete clay separation. The subject was carefully investigated in this laboratory, some years ago, by the late Dr William Beam, who worked out modifications of the usual method with entirely satisfactory results. The essence of Beam's method lies in the substitution of sodium carbonate for ammonia as the deflocculating agent, and after using it for about 1000 samples, we can confidently recommend it for general use.

Beam's paper, entitled "The Mechanical Analysis of Arid Soils," was published in the *Cairo Scientific Journal* for 1911 (pp. 107-119), and was abstracted in the *Experiment Station Record* for 1911 (25, p. 513). Possibly owing to the somewhat limited circulation of the *Cairo Scientific Journal*, few, if any, soil laboratories appear to have tried his method which offers many advantages without any drawbacks as far as we are aware: either simple sedimentation or the centrifuge may be used.

The principal points of difference in the methods now under discussion are as follows: it being understood that by the English method is

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meant that adopted by the Agricultural Education Association (*Journ. of Agric. Science*, 1906, **1**, 470) or *Soil Conditions and Plant Growth* by E. J. Russell, Appendix, p. 151, and the American as described in the *Bulletins* of the Bureau of Soils, Nos. 24 of 1904 and 84 of 1912. The Sudan method is that described by Beam, of which an abbreviated description is given in this paper (pp. 295-297).

Table I.

	English	American	Sudan (sedimentation)	Sudan (centrifuge)
Weight of soil used	10 gms.	5 gms. of fraction less than 2 mm.	2.5 or 5 gms. of fraction less than 1 mm.	2.5 or 5 gms. of fraction less than 1 mm.
Preliminary treatment with acid	yes	no	no	no
Deflocculating agent	ammonia	ammonia	sodium carbonate	sodium carbonate
Time required in shaking machine	not shaken	7 hours	2 hours or less	2 hours or less
Manner of subsequently breaking up	rubber pestle	jet of water	camel-hair brush	rubber-tipped glass rod
Method of separation	gravity	centrifuge	gravity	centrifuge
Time required for clay separation in case of heavy soil	30 to 50 days (one decantation a day)	40 to 50 centrifugings	4 to 6 days (two decantations a day)	12 to 20 centrifugings

The very large saving in time effected by the use of Beam's method is shown in the last line of the table.

### 2. CLASSIFICATION OF PARTICLES.

It is unfortunate that in the different methods here dealt with, the same system of classification of soil particles is not used, the figures for the different fractions being as given in Table II below.

In order to compare the methods, we have adapted the American method to separate clay particles from 0.002 downward by using the same speed of centrifuge and length of liquid column as in our own.

Table II.

Method	English	American	Sudan
Gravel and stones	above 1 mm.	1-2 mm.	above 2 mm.
Coarse sand	1-0.2	1-0.05	2.0-0.2
Fine sand	0.2-0.04		0.2-0.02
Coarse silt	0.04-0.01	0.05-0.005	0.02-0.002
Fine silt	0.01-0.002		
Clay	below 0.002	below 0.005	below 0.002

Microscopic examination of the clay separated by the American and Sudan methods confirmed that the clay fractions were then of the same size particles. The details for the separation methods of the deflocculated clay are as follows:

*Ammonia deflocculation.*

Sedimentation (English) ...	Height of column: 8.6 cms. time: 24 hours.
Centrifugal (American) ...	Radius (from centre of centrifuge to upper surface of liquid): 10 cms.; time: 6 minutes at 840 r.p.m.

*Sodium carbonate deflocculation (Sudan).*

Sedimentation ... ..	Height of column: 10 cms.; time: 8 hours.
Centrifugal ... ..	Radius: 10 cms.; time: 6 minutes at 840 r.p.m.

The two last give equivalent treatment, since a rotation of 840 r.p.m. at a radius of 10 cms. gives a centrifugal force 79.2 times that of gravity: this, divided into 8 hours (the period allowed for gravity only), gives 6.05 minutes.

The English method prescribes a longer period for sedimentation and this is reflected in the results as will be seen below.

### 3. DETAILS OF THE SUDAN SEDIMENTATION METHOD.

One hundred gms. of the soil dried at 100° C. are sieved, the sieve having round holes 2 mm. in diameter; a wooden rolling-pin is used to break up large aggregates and the particles left on the sieve are washed, dried and weighed as "stones and gravel" (above 2 mm.).

The portion passing through the sieve is then similarly sieved through a 1 mm. sieve, the retained portion being washed, dried and weighed as part of the "coarse sand" fraction (2.0-0.02 mm.).

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Five gms. of the portion passing the 1 mm. sieve (or 2.5 gms. when much clay is known to be present) are weighed out and shaken for about 2 hours with 100 c.c. of 0.2 per cent. sodium carbonate solution<sup>1</sup>.

The soil is transferred to a beaker and water added to a height of 10 cms. and the liquid having been thoroughly mixed and allowed to stand at least 16 hours, the clay suspension is poured off and replaced by 0.05 per cent. sodium carbonate solution. This, and the subsequent subsidences, are alternately of eight hours and over-night. After the third or fourth decantation, the residue is puddled with a brush, care being taken to wash all the soil adherent to the brush back into the beaker. After all the clay has been removed—six to nine decantations being usually sufficient—the residue is washed into a smaller weighed beaker with water, allowed to stand eight hours, the water decanted and the contents of the beaker evaporated to dryness, dried at 100° C. for about 12 hours and weighed. This portion contains all particles smaller than 1 mm. and larger than 0.002 mm. and the clay is, therefore, determined by difference.

The removal of the silt is carried out by washing the particles from 1.0 mm. to 0.002 mm. diameter into a beaker and decanting after a subsidence of 7½ minutes in a 10 cms. column of water. After the silt has been thus removed the remaining particles are washed back into the original weighed beaker, dried as before and weighed—the silt being determined by difference.

The remaining soil, consisting of particles 1.0 mm. to 0.02 mm. in diameter, are separated by means of an appropriate wire sieve into “coarse sand” and “fine sand,” each portion being weighed separately and the amount of coarse sand added to that previously found. There is usually a slight loss from the operation from material adhering to the sieve and beaker so that if the “fine sand” is determined by difference all the loss is reflected in the “fine sand.”

All results are expressed as percentages on the soil dried at 100° C.

We ourselves prefer this method to any other. Although a batch of samples usually requires seven to ten days, the amount of attention required is very small, and one assistant can easily keep 40 samples going at once and still have time for a good deal of additional routine work.

Duplicate determinations of the clay agree within a few per cent. and are often quite close, as is shown by the following samples:

<sup>1</sup> Bean states that this preliminary shaking may be replaced by puddling with a camel-hair brush with a little sodium carbonate solution.

Table III.

Sample	Repetition analyses	Mean	Maximum difference
5238	12.4, 13.0	12.7	0.6
5371	30.9, 31.4, 29.9, 30.2	30.6	1.5
5379	39.4, 38.7, 42.4, 39.9	40.1	3.7
5350	57.4, 60.2, 57.6	58.4	2.8
5460	63.8, 66.1, 64.4, 64.6	64.7	2.3
5755	59.2, 59.1	59.2	0.1

A point of practical interest is that the period of shaking is of little importance and can be dispensed with altogether if desired: this is an advantage which the American method does not possess, the period of shaking having an important influence on the result<sup>1</sup>.

The following figures show that 7 hours' shaking gives the same results for clay percentage as 2 hours:

Table IV.

Sample No.	clay 2 hours shaking	clay 7 hours shaking	Difference
5238	12.7	13.2	0.5
5371	30.6	(29.2) (30.0) 29.6	1.0
5379	40.1	(39.1) (42.0) 40.4 (40.1)	0.3
5460	64.7	(64.9) (64.1) 64.5	0.2
5667	57.2	(55.6) (56.0) 55.8	1.0

#### 4. SUDAN CENTRIFUGAL METHOD.

The preliminary treatment of the sample is the same as that described in the preceding paragraph. After the 2 hours' shaking, the contents of the bottle are washed into the centrifuge tubes and the liquid made up to the 10 cms. mark with water. The clay suspension is then centrifuged for 8 to 10 minutes, the suspended clay poured off, and the soil at the bottom of the tube brought into suspension with .05 per cent. sodium carbonate solution by means of a rubber tipped glass rod, filled

<sup>1</sup> The shaker referred to in *U. S. Bulletin* No. 84 is prescribed to run at a speed of 100 shakes per minute: as our machine only runs at 65 per minute, we have increased the time of shaking in proportion where necessary.



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up to the mark and again centrifuged. This and the subsequent centrifugings are for six minutes (see end of section 2), the first one being longer in order to prevent any silt particles being held up by the thick clay suspension. The number of the centrifugings required is usually between 12 and 20 according to the amount of clay present.

The portion left in the centrifugal tubes after the operation consists of particles 1.0 mm. to 0.002 mm. These are washed with water into a weighed beaker and allowed to stand until the liquid is clear. The liquid is decanted and the soil dried at 100° C. for 12 hours and weighed. The clay is then determined by difference.

The separation of the other soil fractions is exactly the same as in the Sudan sedimentation method.

The next table shows that the results obtained agree well amongst themselves and also with those obtained by the Sudan sedimentation method.

Table V.

Sample	% clay by sedimentation method	% clay by centrifugal method	Difference
5238	12.7	{11.7} {12.7} 12.2	0.5
5379	40.1	{39.9} {39.4} 39.7	0.4
5667	57.2	{55.3} {57.5} 56.4	0.8
5460	64.7	{63.8} {65.6} 64.7	0.0

This method allows of a batch of samples being completed in much less time than the sedimentation process, but as it requires almost undivided attention, we ourselves much prefer the latter.

### 5. AMERICAN METHOD.

As will be seen from Table I, the essential points of difference between this and the Sudan centrifugal method are in the use of ammonia as the deflocculating agent and the longer period of shaking required. In Bureau of Soils *Bulletin* No. 24, a large number of results are given showing the influence of the time of shaking on the results and we give below some of our own which confirm this. There is a very considerable objection to the method on this account, as it might be necessary to standardise a shaker in order to obtain consistent results: the Sudan

method appears, as stated above, quite free from this disadvantage. If the time of shaking prescribed (7 hours at 100 shakes per minute, or over 10 hours in our machine) is used, the results agree well with our own method.

We, therefore, do not confirm Beam's statement, that the American method fails to separate a considerable proportion of the clay.

Table VI.

Sample	American method, shaking for				Difference between the 14 hours and Sudan centrifugal method
	2 hours	7 hours	14 hours	28 hours	
5371	{25.6} {22.4} 24.0	—	{29.6} {29.7} 29.7	{30.6} {31.6} 31.1	0.1
5379	{32.4} {32.0} 32.2	{38.0} {36.8} 37.4	{39.8} {40.3} 39.9	{40.8} {41.6} 41.2	0.2
5667	{44.8} {36.9} 41.9	{53.9} {53.1} 53.5	—	{55.6} {56.4} 56.0	—
5460	{48.1} {48.2} 48.2	{60.0} {55.2} 57.6	{65.7} {64.9} 65.3	—	0.6

The great saving of time in the Sudan method combined with the removal of the uncertainty involved by a rigidly prescribed period of shaking are solid advantages in its favour, if a centrifugal method is to be used.

#### 6. ENGLISH METHOD.

The principal points of difference between this and our sedimentation method lie in (*a*) the preliminary treatment with acid, (*b*) the time allowed for sedimentation, (*c*) the height of column of liquid, and (*d*) the use of ammonia instead of sodium carbonate.

With regard to the last, the experiments recorded in Table VI show that ammonia can be used to effect a complete separation of the clay provided that the mechanical treatment is sufficiently energetic.

The preliminary treatment with acid introduces a complication as there is no doubt that dilute acid is capable of dissolving material, which as far as size of particles is concerned, should be considered clay. Thus in one experiment, fifth-normal hydrochloric acid was found to dissolve 0.3 gm. from a 5 gms. sample of purified clay when left in contact with it for about 4 hours. The result is that where a soil contains a high proportion of fine material soluble in dilute acid, the amount of clay as subsequently determined may be less than when there is no preliminary

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acid treatment. This is shown by the figures given a little later (Table IX) for clay found by the Sudan sedimentation method as usually carried out and by the same method in which the clay separation is preceded by acid treatment as in the English method. It will be seen that the two heavy clay samples yield 2 or 3 per cent. of clay to the acid.

The possible solubility of the clay fraction in dilute acid is also of importance as it is the custom in some laboratories using the English method to flocculate the bulky clay suspensions with dilute acid; the precipitated clay thus often remains in contact with the acid for several weeks. As demonstrating the solvent power of dilute acid, some purified clay was allowed to remain in contact with twentieth-normal hydrochloric acid. The clay had previously been precipitated with dilute acid and left in contact with it for some time and then freed from it by prolonged dialysis until the conductivity of the suspension was negligible. Thus purified, it lost 7 per cent. to the acid in three days and 12.5 per cent. in 20 days. This doubtless explains the discrepancies we found in determining the clay indirectly by difference and directly after deflocculation with acid in the same sample: three examples are given below:

Table VII.

Sample	Clay determined		Difference
	By difference	By direct weighing after deflocculation by acid	
5667	{52.3} {51.8} 52.1	{43.5} {44.5} 44.0	8.1
5660	{51.6} {50.8} 51.2	{46.6} {42.7} 44.7	6.5
5460	{56.9} {58.4} 57.7	{51.5} {53.5} 52.5	5.2

We therefore discarded the use of acid for flocculation and either kept the whole of the suspension or determined the clay by difference. That this is satisfactory is shown by the following results obtained for the clay found by the Sudan sedimentation method in three samples which had first been treated with acid as in the English method. For the direct determination, a simple correction had to be applied for the sodium carbonate present which was weighed with the clay. Sodium

carbonate was used instead of ammonia for this test on account of the great saving in time effected.

Table VIII.

Sample	Clay by direct determination	Clay by difference	Difference
5371	29.5	29.9	0.4
5379	40.0	39.6	0.4
5460	61.3	60.6	0.7

Apart from this, there appears satisfactory agreement between the English method and our own when allowance is made for the difference in time of sedimentation and height of the column of liquid. The next table gives the results on four samples obtained by (1) the English method, (2) the Sudan method, (3) the Sudan method modified according to English practice by a preliminary acid treatment and eight hours sedimentation, and (4) the Sudan method preceded by acid treatment, 24 hours sedimentation and a column 8.6 cms. high.

Table IX.

Sample	% soluble in dilute acid	Clay by Sudan sedimentation method	Clay by Sudan sedimentation method		English method*	Difference between last two columns
			preceded by acid treatment	but 24 hours sedimentation using an 8.6 cms. column		
5371	{6.7} {6.7} 6.7	30.6	{29.9} {30.5} 30.2	{26.8} {27.2} 27.6	{28.1} {29.2} 27.6	0.0
5379	{6.9} {7.4} 7.2	40.1	{39.6} {39.8} 39.7	{38.1} {37.9} 38.0	{36.4} {37.1} 36.8	1.2
5350	{8.5} {8.6} 8.6	58.4	{55.2} {56.6} 55.9	{53.3} {52.8} 53.1	{55.2} {53.1} 54.2	1.1
5460	{5.8} {6.0} 5.9	64.7	{60.6} {61.7} 61.1	{59.6} {58.2} 58.9	{56.9} {58.4} 57.7	1.1

\* In order to compare these results with those obtained by the Sudan and American methods the clay was dried at 100° C. and not ignited, since ignition involves, besides humus, loss of combined water which in heavy clay soils may amount to 6-8 % on the original soil: the humus content of these soils was very low, less than 1 %.

From the last two columns it will be seen that if the other conditions (time of sedimentation, acid treatment and height of column) are the

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same, the English method gives the same results as our own involving the use of sodium carbonate. Here again, however, the very great saving in time effected by this deflocculating agent leaves no doubt as to which is the most convenient method. Another disadvantage accompanying the use of the English method is the large quantity of distilled water required for the sedimentation: a heavy clay soil requires 16–18 litres for the English method as against 3–4 litres for our own. It is perhaps of interest to record the following results showing the effect of increasing the time of sedimentation from 8 to 24 hours. These determinations were carried out by the Sudan sedimentation method and required 10 days as against five when the period of sedimentation is 8 hours.

Table X.

Sample	8 hours sedimenta- tion	24 hours sedimenta- tion	Difference
5371	30.6	$\left. \begin{matrix} 26.0 \\ 25.2 \end{matrix} \right\} 25.6$	5.0
5379	40.1	$\left. \begin{matrix} 37.6 \\ 37.2 \end{matrix} \right\} 37.4$	2.7
5460	64.7	$\left. \begin{matrix} 61.2 \\ 61.6 \end{matrix} \right\} 61.4$	3.3

The differences are rather larger than indicated by Beam, who gives it as about 2 per cent.

### SUMMARY.

1. The Sudan method for clay determination worked out by Dr Beam in 1911 has been critically examined and compared with the methods in general use in England and America.

2. The essential points of the method lie in (*a*) the use of sodium carbonate instead of ammonia as the deflocculating agent, and (*b*) the use of a camel-hair brush for puddling the clay. It differs from the English (and resembles the American) method in that no preliminary acid treatment is used, the height of the sedimentation column is 10 cms. and the time of subsidence 8 hours.

3. It can be applied to a sedimentation or centrifugal process: the results agree well with each other and with those obtained by the American method.

4. When modified by inclusion of acid treatment, by increasing the time of sedimentation from 8 to 24 hours and reducing the height of

the column to 8.6 cms., the results agree well with those obtained by the English method.

5. Treatment with acid either in the preliminary operations or for deflocculating clay suspensions may lead to uncertainty in the results in some cases as it causes loss of "clay" by solution.

6. The Sudan sedimentation method can be carried out in about one-eighth the time required for the English method, and the centrifugal in about one-third the time required for the American. For general use the sedimentation method is preferred as requiring less attention.

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## THE DIGESTIBILITY OF OAT AND TARE SILAGE.

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DURING the last decade the production of silage has, in various parts of the country, become a matter of considerable importance, and many scientific investigations in connection with this practice have been carried out. The bulk of this work has been directed towards the study of the changes undergone by the crop in the silo, together with the determination of the character and the amount of the losses which occur. Little or no attention has been given to the question of the digestibility of silage, although it is of obvious interest to determine how the digestibilities of its ingredients compare with those of the green crop from which it has been made. The investigation to be described in this communication is preliminary to one of larger scope, which will have for its object the comparison of the digestibilities of the green fodder, hay and silage of the oat and tare crop. A knowledge of these comparative data, when considered in conjunction with the available information regarding the losses sustained by the crop in the making of hay and silage, will enable the question of the relative economy of hay and silage production to be settled.

### QUALITY OF THE SILAGE USED IN THE DIGESTION TRIAL.

In view of the fact that the digestibility of silage may be influenced in some degree by such factors as time of cutting, character and extent of the changes which it has undergone in the silo, etc., it is of importance to note the conditions under which the fodder was produced.

Mr Arthur Amos has kindly furnished us with the following details:

"The silage was made from an oat and tare crop in which, though sown in equal proportion, the tares largely predominated at time of cutting. The crop was cut on July 12, 1920, wilted one day and chaffed for silage without having been wetted by rain. At time of filling, therefore, the crop was fairly dry and contained from 30 to 35 per cent. of dry matter. When used (Jan. 31 to Feb. 22, 1921) the percentage of dry matter varied between 33 and 37 per cent. The maximum temperatures in this part

of the silo varied between 33 and 36.2° C. The character of the silage was really good without being of the finest quality; it had the characteristic brownish colour of silage made from wilted oats and tares and the not unpleasant, yet characteristic, odour associated with the same."

#### EXPERIMENTAL EQUIPMENT.

The equipment employed in this trial for collecting faeces and urine separately differed in many respects from the means commonly used and was devised by Mr E. T. Halnan, from whom the following account has been received:

"The feeding stalls in which the sheep were kept during the trial consisted of iron cages of the usual Lehmann type, modified in order to render them suitable for the harness used in the experiment. Previous work with digestion harness has shown that both the urine funnel and the faeces bag are frequent sources of trouble, and the cage and harness used in this experiment were designed to eliminate both these sources of difficulty. The harness consisted, in its essentials, of a rubbered canvas sheet fixed by suitable means to the hindquarters of the sheep. The lower end of this sheet was weighted and could thus hang freely over the end of the floor of the cage, the weight keeping the sheet taut whether the sheep was in a standing or a lying position. The solid excreta were, by this means, directed to the rear outside of the cage and fell down a funnel shaped shoot attached to the cage into a receptacle below. A portion of the floor of the cage was drilled with holes and two parallel metal guides were fixed to the under surface of the two sides of this portion. The guides acted as a support for a metal funnel and allowed the latter to be withdrawn easily for cleaning purposes. The urine of the sheep drained through the perforated portion of the cage floor into the funnel and was caught in a bottle placed underneath the neck of the funnel. By raising slightly the rear portion of the cage, the floor was given a slight tilt which effectually prevented the possibility of contamination of the rubbered canvas sheet by the urine."

#### PLAN OF EXPERIMENT.

Owing to the possible inadvisability of keeping sheep over a period of three or four weeks on a large ration consisting solely of silage, it was decided to feed a basal ration consisting of chaffed meadow hay and a little linseed cake. Consequently, it was necessary to include two periods in the trial. The length of the experimental periods was 14 days, and these were preceded by a preliminary period of 7 days. The trial was carried out in duplicate, two well-behaved wethered sheep being used for the purpose. Both sheep showed an initial disinclination to eat the silage; this objection was, however, speedily overcome, and indeed, towards the end of the silage period, they had become noticeably fond of the fodder. Unfortunately, in this period, Sheep I left a small amount of hay residue, and this had to be allowed for in the final calculation of the digestion coefficients for this animal.



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The sheep were weighed at the beginning and end of each period. The care of the animals was in the hands of Mr F. J. Aylett, who also carried out the analytical work in connection with the investigation.

### SAMPLING AND ANALYSIS OF FOODSTUFFS.

*Meadow hay.* The daily rations for the whole period were weighed out from the bulk into paper bags, after drawing a representative sample by quartering for complete analysis. The moisture content of the hay was determined at the same time.

*Linseed cake.* A period composite sample for analysis was made up by reserving a definite weight each day.

*Silage.* Biweekly composite samples were made up and nitrogen and moisture determinations were carried out on the fresh material of these samples. The moisture content was found by drying down aliquot portions of the samples in the steam oven, and the dried residues were preserved in an air-tight bottle. At the end of the period, these residues were mixed, finely ground and air dried. A complete analysis was then carried out.

### SAMPLING AND ANALYSIS OF FAECES AND URINE.

The fresh faeces were weighed daily, aliquot portions being reserved in air-tight bottles for making up biweekly composite samples. The nitrogen content of the fresh minced faeces was determined in triplicate. The percentage of moisture was ascertained by drying down aliquot portions of the composite samples at 65° C. in an air oven. From the dry residues was made up the period composite sample for complete analysis. It will be noted that no determinations of true protein were made, nor was it considered essential to correct the protein digestion coefficients by carrying out estimations of the metabolic nitrogen of the faeces.

The output of urine and its nitrogen content were followed throughout the periods. A preliminary determination of the pentosan content of the urine in the silage period showed this to be very small, and consequently no further determinations of this ingredient were made.

No record was kept of water consumption.

Table I. *Record of rations.*

Period	Daily ration
I	1000 gm. oat and tare silage
..	500 gm. chopped meadow hay
	100 gm. linseed cake (broken to size of nuts)
II	500 gm. meadow hay
	100 gm. linseed cake

Table II. *Average composition of foodstuffs (calculated to dry matter).*

	Meadow hay %	Linseed cake %	Silage %	Hay residues from Sheep I in Period I %
Crude protein ... ..	9.05	31.31	16.23	10.83
Ether extract ... ..	4.28	10.77	4.25	4.00
Nitrogen-free extractives	50.26	41.49	37.30	46.72
Crude fibre ... ..	28.95	9.90	32.98	30.33
Ash ... ..	7.46	6.53	9.24	8.12
Average amount of dry matter in hay per day during both periods				= 443 gm.
" " " cake "	"	"	"	= 89.0 "
" " " silage " in Period I	"	"	"	= 346.4 "
" " " hay residues from Sheep I in Period I	"	"	"	= 50.8 "

Table III. *Average weight and composition of faeces.*

	Period I		Period II	
	Sheep I	Sheep II	Sheep I	Sheep II
Weight of fresh faeces daily, gm.	826	973	450	419
Weight of dry matter daily, gm.	323	339	189	185

*Composition of dry matter.*

	Period I		Period II	
	Sheep I	Sheep II	Sheep I	Sheep II
	%	%	%	%
Crude protein* ... ..	13.04	12.06	14.20	13.69
Ether extract ... ..	3.25	2.99	4.16	4.00
Nitrogen-free extractives	40.53	41.80	42.24	42.22
Crude fibre ... ..	30.53	30.33	24.77	25.06
Ash ... ..	12.65	12.82	14.63	14.94
* Crude protein as determined on fresh faeces	5.41	4.53	6.25	6.23

Table IV. *Digestibility of Basal ration (Period II).*

Average daily ration: 500 gm. meadow hay + 100 gm. linseed cake.

*Sheep I.*

	Total dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	Nitrogen- free ex- tractives gm.	Crude fibre gm.	Ash gm.
Consumed:							
Meadow hay ... ..	443.0	410.0	40.1	19.0	222.6	128.3	33.0
Linseed cake ... ..	89.9	84.0	28.1	9.7	37.3	8.9	5.9
Total ... ..	532.9	494.0	68.2	28.7	259.9	137.2	38.9
Voided ... ..	189.0	161.3	28.1*	7.9	79.8	46.8	27.7
Digested... ..	343.9	332.7	40.1	20.8	180.1	90.4	11.2
Digestion coefficients, %	64.53	67.35	58.80	72.48	69.30	65.90	28.79

*Sheep II.*

Consumed (as above) ...	532.9	494.0	68.2	28.7	259.9	137.2	38.9
Voided ... ..	185.0	157.3	26.1*	7.6	78.0	46.4	27.7
Digested... ..	347.9	336.7	42.1	21.1	181.9	90.8	11.2
Digestion coefficients, %	65.28	68.17	61.74	73.52	69.99	66.20	28.79
Mean digestion coefficients, %	64.9	67.8	60.3	73.00	69.6	66.1	28.8

\* Calculated on fresh faeces.

### 308      *The Digestibility of Oat and Tare Silage*

It will be noted that the results obtained in this period are very satisfactory, the agreement throughout between the digestion coefficients for the two sheep being excellent.

Table V. *Period I (basal ration + silage).*

Average daily ration: 500 gm. meadow hay + 100 gm. linseed cake + 1000 gm. silage.

<i>Sheep I.</i>							
	Total dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	Nitrogen-free extractives gm.	Crude fibre gm.	Ash gm.
Consumed:							
Meadow hay ...	443.0	410.0	40.1	19.0	222.6	128.3	33.0
Linseed cake ...	89.9	84.0	28.1	9.7	37.3	8.9	5.9
Silage ...	346.4	314.4	56.2*	14.7	129.2	114.3	32.0
Total ...	879.3	808.4	124.4	43.4	389.1	251.5	70.9
Voided, total ...	323.0	282.1	44.7†	10.5	130.9	98.6	40.9
From basal ration ...	189.0	161.3	28.1	7.9	79.8	46.8	27.7
Indigestible matter from ‡ 50.8 gm. dry hay residue ...	21.0	19.0	2.4	1.0	9.0	6.6	2.9
Corrected indigestible matter from basal ration ...	167.1	142.3	25.7	6.9	70.8	40.2	24.8
From silage ...	155.9	139.8	19.0	3.6	60.1	58.4	16.1
Digested from silage ...	190.5	174.6	37.2	11.1	69.1	55.9	15.9
Digestion coefficients of silage, % ...	55.00	55.53	66.19	75.51	53.49	48.90	49.70
<i>Sheep II.</i>							
Consumed (as above) ...	879.3	808.4	124.4	43.4	389.1	251.5	70.9
Voided, total ...	339.0	295.5	44.0†	10.2	141.5	102.9	43.5
From basal ration ...	185.0	157.3	26.1	7.6	78.0	46.4	27.7
From silage ...	154.0	138.2	17.9	2.6	63.5	56.5	15.8
Digested from silage ...	192.4	176.2	38.3	12.1	65.7	57.8	16.2
Digestion coefficients of silage, % ...	55.54	56.04	68.15	82.31	50.85	50.57	50.63
Mean digestion coefficients of silage, % ...	55.3	55.8	67.2	78.9	52.2	49.7	50.2

\* The figure obtained on the basis of N determinations on the *fresh* silage was 56.4 gm.

† Calculated on fresh faeces. In every case a slight loss of nitrogen occurred on drying down the faeces at 65° C.

‡ Correction introduced to allow for hay residues left by Sheep I in this period. In making the correction the following average digestion coefficients calculated from Kellner's figures for meadow hay were employed: crude protein, 55.7 per cent.; ether extract, 50 per cent.; nitrogen-free extractives, 62.1 per cent.; crude fibre, 57.0 per cent. The ash digestibility of the hay residue was assumed to be the same as that for Sheep I in the basal period.

The agreement between the results for the two sheep in this period, whilst not quite so good as that obtained in the basal period, is still very satisfactory, the only discordant result being that obtained for the digestibility of the silage ether extract. Such lack of harmony in the case of ether extract digestibilities is, however, of common occurrence in digestion trials, especially in the case of coarse fodders, where the amount of this ingredient forms such an inappreciable fraction of the total ration. A slight correction for the digestibility coefficient of the

ether extract of the silage is furthermore necessary, since the analytical work was carried out on the *dried* silage and faeces; the figure obtained does not therefore take into account the digestibility of the volatile organic acids of the silage. It may be assumed, however, that these are fully absorbed by the animal.

The high percentage digestibility of silage protein, as compared with that of the basal ration, is noteworthy, as is the comparison of the ash digestibilities in the two periods. The digestibility of the total dry matter of the silage, however, is appreciably lower than that of the dry matter of the basal ration.

By utilising the digestibility coefficients obtained in this period, it is possible to calculate the percentages of digestible nutrients in the oat and tare silage (dry matter).

	%
Crude protein ... ..	10.91
Ether extract ... ..	3.35
Nitrogen-free extractives ... ..	19.47
Crude fibre ... ..	16.39

Production starch equivalent (Kellner) per 100 lb. *dry* silage = 33.4  
 " " " " " " *fresh* " = 11.57

A fuller discussion of the significance of the data obtained in this trial will be deferred until the completion of the comparative digestibility work on the green fodder, hay and silage of the oat and tare crop, when it is hoped that data will be available which will enable the absolute losses of digestible nutrients suffered by the green fodder in hay and silage production to be calculated.

Table VI. *Nitrogen balances during trial and weights of sheep.*

Period	N consumed. Average per day gm.	N voided			N retained (+) by sheep. Average per day gm.
		In faeces gm.	In urine gm.	Total gm.	
I. Hay + linseed cake + silage:					
Sheep I... ..	19.02	7.15	8.72	15.87	+3.15
Sheep II ... ..	19.90	7.04	9.79	16.83	+3.07
II. Hay + linseed cake:					
Sheep I... ..	10.91	4.50	4.62	9.12	+1.79
Sheep II ... ..	10.91	4.17	5.79	9.96	+0.95
		<i>Sheep I</i>		<i>Sheep II</i>	
		st. lb.		st. lb.	
Period I { Jan. 31, 1921	8 8 }	- 1 3/4 lb.		9 4 }	+ 6 lb.
Period II { Feb. 22, "	8 6 1/2 }	- 1 1/4 "		9 10 }	- 9 "
Period II { March 15, "	8 5 }			9 1 }	

It will be noted that even in the basal period, both sheep showed a slight positive nitrogen balance. The loss in weight suffered by the sheep in this period was not very appreciable.

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## THE SURVIVAL OF MOTILITY IN MAMMALIAN SPERMATOOZOA<sup>1</sup>.

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ONE of the problems in breeding which awaits a satisfactory solution is distant insemination. This matter has received attention from certain physiologists from very early times. Spallanzani<sup>(1)</sup>, with the genius which characterised his vision of all physiological problems, recognised its importance and made certain model experiments in artificial insemination.

The subject has been pursued in Russia where important studs are separated from breeders by long distances, and the most complete summary of our knowledge is contained in a monograph by Iwanoff<sup>(2)</sup>, entitled "*De la fécondation artificielle chez les mammifères.*" In this country Heape<sup>(3)</sup> has pointed out its importance.

Practically all the work up to now has been in the nature of experiments on the insemination of animals by means of sperm collected and introduced almost immediately after coition. In some instances an hour or two has elapsed between ejaculation and the introduction of the sperm into a third animal.

Apart from simple artificial insemination, which has now been practised with complete success in man and the lower animals for many years, and the technique of which has been worked out with considerable thoroughness, the question of distant insemination, which is of great practical importance, from the breeder's point of view, is almost untouched. My attention was directed to the problem by Dr F. H. A. Marshall, who had already made certain preliminary experiments in this direction<sup>(4)</sup>. I undertook to ascertain to what extent physiological environment influenced the survival of spermatozoa *in vitro*.

It is obvious that were one in a position to send sperm to a distance and successfully inseminate, the cost of breeding would be very greatly reduced and the standard materially raised.

<sup>1</sup> The experiments described in this paper were performed in the Institute for the Study of Animal Nutrition, School of Agriculture, Cambridge University.

That the problem should be capable of solution seems to be *a priori* certain. Given suitable environment, spermatozoa ought to be perfectly capable of surviving outside the testicle for long periods of time. An extreme example of this is that of the sperm of the drone, which remains fertile in the spermatheca of the queen bee for four and even five years (Cheshire<sup>(5)</sup>). Indeed we have had practically effected in bee-keeping what is so much desired in animal breeding, viz. the means of transport of sperm to distant parts. The means, of course, in this case is the queen herself.

It is also well known that spermatozoa remain capable of fertilising within the mammalian female organism for long periods. Haussman<sup>(6)</sup> estimated that they lived eight days; Dührsenn<sup>(7)</sup> three weeks. The latest estimate by Pryll<sup>(8)</sup> confirms both these. As the result of the examination of cohabitation curves, the average duration of life of human spermatozoa was made out to be six to ten days, while in certain cases the spermatozoa were viable at the end of 20 days.

When it comes to the use of physiological solutions for preserving the life of spermatozoa, there is very little information available. No such exact investigation of the behaviour of mammalian spermatozoa has been made as that of Cohn<sup>(9)</sup> with *arbacia* sperm<sup>1</sup>. Cohn measured the activity of these cells, not only by observing motility, but by carbon dioxide production and the changes which this produced on the hydrogen ion concentration of the sea-water in which they were suspended. He was able to show that spermatozoa were very sensitive to changes in acidity. In sea-water, which varies at Wood's Hole during July and August from  $P_H = 7.95$  to  $8.15$ , the sperms live for a long time. In water more acid than  $P_H = 7.6$  the cells were rapidly destroyed.

A certain amount of information may be obtained from the work on tissue culture and tissue survival by Harrison<sup>(10)</sup>, Carrel<sup>(11)</sup>, Burrows<sup>(12)</sup> and others. In this type of tissue survival the presence of plasma seems almost essential. Ciaccio<sup>(13)</sup> states that he did not get so good growth in media which did not contain blood plasma. A mixture of plasma and Ringer's solution seems to be most effectual. As most of the experiments

<sup>1</sup> An exhaustive report by Jinshin Yamane (*Journ. Coll. of Agric. Hokkaido Imp. Univ.* 1921, 9, 161) has appeared since this paper was written. The effect of many salts on the motility of horse spermatozoa was examined. Although the author seems to have appreciated the importance of the hydrogen ion concentration on motility no attempt was made to control this factor in his experiments. His results are therefore deprived of much of their value. He details certain successful insemination experiments with semen diluted with physiological saline solution and glucose. The semen was used at once after dilution. It was collected in a condom made from a pig's bladder.

which have been done on tissue survival have been made for the purpose of observing growth, the preparations have been kept at body temperature. It has been observed, however, that for transplantation experiments it is better to preserve the tissue at a low temperature. The difference between the viability of mouse cancer kept at  $+2^{\circ}\text{C}$ . and at  $37^{\circ}\text{C}$ . at the end of quite a short period is very great (Gye(14)). Iwanoff describes certain experiments which show that sperm survives longer when kept at a low temperature. In most of his experiments the spermatozoa were contained in the isolated testicle, a fact which, as will be shown in my own experiments, has an important influence on survival. Iwanoff states that the testicle may become partly putrid, but that in the interior motile sperms will be found. Sperm may be frozen and lowered to  $-15^{\circ}\text{C}$ . When thawed the cells will show feeble movement.

The oxygenation of the sperm suspension has been studied by Ochi(15) and by Sato(16). They state that oxygen in moderate amounts is helpful in preserving the life of the cells, but that an excess is injurious. The experiments of these observers are not altogether satisfactory, for it will be found that the means employed of regulating the neutrality of the medium were not such as would give good comparative results. In surviving tissue Loeb and Fleischer(17) found that those particles of tissue which were nearest an atmosphere of pure oxygen grew most vigorously. Gye's(18) experience with the transplantation of carcinoma tissue corresponds with this. He finds it essential that the carcinoma emulsion should be very well supplied with oxygen if it is to preserve its viability for any length of time.

A systematic examination of the concentration and character of the solution in which the sperm is to be preserved has not been undertaken.

Iwanoff states that Locke's solution is satisfactory and finds that the spermatozoa preserve their motility in solutions of salts as different as sodium chloride, barium chloride and potassium nitrate. The length of time and temperature are not stated.

Both Ochi and Sato confirm these results, and add a statement, which from my experiments seems to be important. The medium is improved by an addition of an isotonic solution of glucose. Poyarkov(19) had previously shown that those sperms preserved in a mixture of nine parts of isotonic glucose and one part of isotonic salt solution were more motile than when smaller concentrations of glucose were employed.

## EXPERIMENTAL.

The object of the present experiments was to ascertain the composition of the solution and the other conditions under which rabbit's sperm was best preserved in a state of motility. Certain experiments were also undertaken to find out if sperm preserved under these conditions was capable of effecting pregnancy. It is to be regretted that these experiments are incomplete, but it is hoped that the data presented may be of service to other investigators in this important field.

It appears that in the preparation of a physiological solution in which spermatozoa are to be preserved, at least two conditions are necessary. One is that the reaction of the fluid should be as near that of normal blood as possible. Parsons<sup>(20)</sup> and others have shown that this may be taken for man as  $P_{II} = 7.35$ . In the second place, it seems essential that a sufficiency of buffer salts should be present in the solution to prevent any great change in reaction taking place due to metabolism of the cells themselves. I have shown<sup>(21)</sup> that in the preparation of vibriion septique toxine the initial reaction of the medium combined with the presence of sufficient buffer salts to stabilise the reaction had an important effect on the production of the antigen.

As a preliminary, a ready-made Locke's solution was employed, made by dissolving tablets of Burroughs & Wellcome's "Soloids Saline Compound" in the requisite quantity of water. I was anxious to employ a commercial preparation of this sort for in the event of success it would facilitate work being done by breeders.

The composition of the solution furnished by these tablets as given on the container is as follows:

	%
Sodium chloride ...	0.723
Potassium chloride	0.0159
Calcium chloride ...	0.0159
Sodium bicarbonate	0.0079
Glucose ... ..	0.080

For comparison the composition of two of the best known physiological saline solutions is given.

	Locke	Tyrode
Sodium chloride ...	0.9	0.8
Potassium chloride ...	0.042	0.02
Calcium chloride ...	0.024	0.02
Sodium bicarbonate ...	0.01 -0.03	0.10
Magnesium chloride	—	0.01
Sodium acid phosphate	—	0.005
Glucose ... ..	0.1 -0.25	0.10
Water ... ..	100.0	100.0



One of the first questions investigated was the influence of buffering a saline solution so that changes produced by the metabolism of the spermatozoa would have less effect upon them. It is well known that one of the deficiencies in Locke's and similar solutions is the lack of these regulating salts.

It will be noted that the content of the Burroughs & Wellcome soloids in all salts is somewhat lower than in the Locke and Tyrode solutions and that Locke's solution is distinguished by its high comparative concentration in potassium chloride.

The general plan of the first experiment reported was to kill a rabbit and immediately remove the two epididymes. These were quickly placed in a short wide test-tube of 25 c.c. capacity containing about 15 c.c. of the solution to be investigated. The tube was corked and placed in a larger tube. This large tube was surrounded by cracked ice contained in a large thermos tube. It was perfectly easy to keep the solution in the inner tube at  $+1.0^{\circ}$  to  $+2.0^{\circ}$  C. for many days with the expenditure of very little ice. The motility of the sperms was observed by taking out a small amount from the cut end of the epididymis and placing it on a cover slip which was inverted in a ring microscopic slide. In some cases the exuded sperm at the bottom of the tube was used. Evaporation was avoided by sealing the cover slip to the ring with vaseline. The preparation was enclosed in a warm stage<sup>1</sup> at  $37^{\circ}$  C. and observed with Zeiss AA and DD, Ocular 5. If the preparation was kept for some hours, it was placed in an incubator at  $37.5^{\circ}$  C. and subsequently examined on a warm stage.

*Experiment 1.* The influence of buffer salts on survival. A solution of Burroughs & Wellcome's soloids was prepared in distilled water. The hydrogen ion concentration of this solution was  $P_H$  6.1<sup>2</sup>. To 100 c.c. of this solution 2.0 c.c. of  $M/5$  acid potassium phosphate solution and 23.0 c.c. of  $M/15$  di-sodium phosphate solution were added. The hydrogen ion concentration of the resulting mixture was  $P_H$  7.4. The epididymes of a rabbit were placed, one in the buffered solution and the other in the unbuffered solution and kept at  $2.0^{\circ}$  C.

24 hours. Both sets of sperms very active. Unbuffered not so active as buffered. After 20 minutes at  $37^{\circ}$  C. both active, buffered especially so.

<sup>1</sup> See Ehrlich, *Encyc. Mikroskop. Tech.* 1903, **2**, p. 719.

<sup>2</sup> The hydrogen ion concentration was determined colorimetrically against standard tubes (see *Medical Research Council Report*, "Reaction of Media," No. 35, 1919). This is of some importance in view of Lovatt Evans' statements regarding the determination of the hydrogen ion concentration of solutions containing carbonates with the hydrogen electrode.

72 hours. In buffered solution many active sperms, majority inactive. Activity lasted an hour at temperatures from 17° C. to 37° C. No sign of activity in the unbuffered solution.

120 hours. Non-buffered sperms all dead. In the buffered solution a certain number of sperms still alive and quite active.

*Experiment 2.* The influence of oxygen on survival. The same solution was employed as before.  $P_H$  of buffered solution, 7.4.  $P_H$  of unbuffered solution, 6.6. Oxygen was passed into both solutions for 15 minutes while the tubes were cooled in ice-water. Immediately after saturation an epididymis was placed in each tube.

48 hours. Examined at 37° C. Buffered specimen had many motile sperms, non-buffered, only a few and not very active.

96 hours. One or two sperms in buffered solution show slight movement. Those in unbuffered are all dead.

*Experiment 3.* As Poyarkov and Sato had shown that survival of spermatozoa was facilitated by the addition of large quantities of glucose to the medium, an experiment was arranged to test this point. A  $M/8$  (5.2 per cent.) solution of glucose was made up (Merek, "Highest Purity"). Equal parts of Burroughs & Wellcome's saline and the glucose solution were mixed. One part of this mixture was buffered as before with sodium and potassium phosphates and the other solution left unbuffered. Epididymes were placed in the two solutions and kept at 2.0° C.

48 hours. All sperms in a high state of activity. The sperms on the microscopic slide kept at room temperature for 5 hours were still very active. The non-buffered solution contained many active sperms, but none of them were so active as those in the buffered solution. Some of the buffered sperms were still active after standing all night at room temperature.

96 hours. Large numbers of active sperms in the buffered solution. None in the non-buffered solution.

120 hours. Few sperms distinctly active.

This experiment seems to show beyond question that the effect of adding glucose to the medium is a beneficial one.

From this time on solutions were prepared from pure salts. The sodium chloride used was a specimen Kahlbaum's fused sodium chloride "For Analysis." The potassium chloride was of the same standard of purity. The calcium chloride was prepared by dissolving a weighed quantity of Iceland spar in an excess of pure hydrochloric acid and evaporating to dryness on the water-bath. The residue was redissolved three

times in water and each time evaporated to dryness. The solution was made up to a definite volume. It was not acid to Congo red paper. Stock solutions of all these salts were made up and the necessary volume used for making up the final solutions. The sodium bicarbonate was always weighed out dry and added to the solution. Freshly distilled water which had been condensed in a tin condenser was used. Except in one experiment it was not redistilled from glass.

*Experiment 4.* The use of Locke's solution with the addition of glucose and oxygen.

50 c.c. of Locke's solution prepared from the above salts was mixed with 50 c.c. of a 5.2 per cent. glucose solution. The mixture had a hydrogen ion concentration of  $P_H$  7.0. To this was added 2.0 c.c. of  $M/5$  acid potassium phosphate and 7.0 c.c. of  $M/5$  di-sodium phosphate. The hydrogen ion concentration of the mixture was  $P_H$  7.4. A portion of this solution was treated with oxygen at room temperature for 10 minutes, was then cooled down and saturated with the gas at ice temperature for 5 minutes. The control solution was not treated with oxygen. An epididymis was placed in each solution and kept at 2° C.

24 hours. Both suspensions very active. The non-oxygenated sperms seem to be more active.

48 hours. Oxygenated sperms quite active. Non-oxygenated sperms, very few show motility.

48 hours. Both active, but the preparations do not seem to be as good as in the previous experiment.

96 hours. Cut open both epididymes and took sperms from the cut surfaces. There is activity in both preparations, but the activity in the oxygenated specimen is decidedly the greater.

144 hours. Movement of the oxygenated sperms quite distinct. Movement in non-oxygenated cells doubtful.

The effect of oxygen on the sperms is, without doubt, to prolong survival. This experiment did not appear to give such good results as the previous one, and as the concentration of potassium both from the potassium chloride and from the potassium phosphate seemed excessive, it was decided to use a Tyrode solution omitting the magnesium chloride.

*Experiment 5.* 100 c.c. of Tyrode's solution was prepared. Owing to the increase in concentration of sodium bicarbonate the hydrogen ion concentration was less than in the solution of Locke.  $P_H$  of Tyrode's solution = 7.8. Added 2.0 c.c. of  $M/5$   $KH_2PO_4$  and 7.0 c.c. of  $M/5$   $Na_2HPO_4$ .  $P_H$  of resulting mixture = 7.5.

Mixed 50 c.c. of the adjusted Tyrode solution and 50 c.c. of  $M/8$  of glucose solution.

As it was uncertain to what extent the buffering action of the tissue of the epididymis affected the results an attempt was made to decide this question. It was important, because in any future practical application of the method pure semen would be used. Iwanoff had also shown that sperms would remain motile within the isolated epididymis for a long time.

In this experiment four tubes were set up. In each one of two was placed one-half of a rabbit's epididymis. In the second two was placed an emulsion made by cutting up the second gland as finely as possible in a very small quantity of Tyrode's solution in a paraffined watch glass.

By careful manipulation the suspension of spermatozoa was transferred to the cooled solution accompanied by as little tissue as possible.

There were therefore four tubes containing sperm as follows:

1. Epididymis + Tyrode, oxygenated.
2. Epididymis + Tyrode, not oxygenated.
3. Sperm + Tyrode oxygenated.
4. Sperm + Tyrode, not oxygenated.

All tubes were packed in ice in thermos flasks 24 hours.

Examined at room temperature Nos. 3 and 4, oxygenated quite active, non-oxygenated not at all active. The slides were placed in the incubator at 38° C. for 30 minutes. The activity of the oxygenated sperms was greater than I had ever seen them. Not a single quiescent spermatozoon could be observed. The non-oxygenated suspension contained a good many active sperms, but activity on the whole was not good.

48 hours. The oxygenated sperms are very active. There is some activity in the non-oxygenated sperms but it is not very great.

72 hours. Activity in oxygenated sperms very much greater.

120 hours. In the oxygenated tube, very few moving slowly. The sperms without oxygen are immobile.

120 hours. Tubes Nos. 1 and 2 containing the pieces of epididymis were now examined. The oxygenated sperm was very active. The non-oxygenated specimen not very active. It would appear from this experiment that Tyrode's solution prepared with a buffer is a better medium for survival than Locke's, and that spermatozoa preserved in the tissue of the gland survive better than when isolated, even when gland and sperm are in the same solution.

*Experiment 6.* The preceding experiment was repeated except that

the tissue tubes were omitted. At the end of 96 hours there was decided and vigorous movement in the oxygenated sperm. In the non-oxygenated specimen a few sperms were moving, but movement was not so vigorous.

*Experiment 7.* As tissue cultures seem to be most successful in a medium containing serum an experiment was made to ascertain the effect of serum on survival.

A rabbit was bled from the carotid under ether anaesthesia and the blood collected in an agar tube (Gardner(23)). The serum was collected and centrifuged at high speed to free from red cells.

To 10 c.c. of an emulsion of sperm in Tyrode's solution and glucose was added 1 c.c. of the serum (the serum was of the same rabbit as the sperm). At 72 hours the sperm was not in any way so active as the control without serum.

*Experiment 8.* The effect of adding an increased quantity of serum was investigated. The concentration of serum was 1--6. At the end of 52 hours it appeared that the spermatozoa in the serum medium were not so active as in the control.

*Experiment 9.* The effect of lowering the concentration of potassium in the medium was further investigated. This was done by replacing the  $M/5$  acid potassium phosphate by a  $M/5$  solution of acid sodium phosphate. The solution used was Tyrode buffered with 2.0 c.c. of  $M/5$   $\text{KH}_2\text{PO}_4$  and 8.0 c.c. of  $M/5$   $\text{Na}_2\text{HPO}_4$ . The final  $\text{P}_\text{H}$  of this solution was 7.5. This was mixed with an equal volume of  $M/8$  glucose. At the end of 72 hours the sperms were quite active. At 96 hours no activity could be made out.

*Experiment 10.* This experiment was performed to ascertain the effect of distilling water from glass in making up Tyrode's solution. There did not appear to be any pronounced effect which could be attributed to the toxicity of traces of tin which might be contained in ordinary distilled water. It was at this point that an error in technique was discovered which had a very pronounced effect on the composition of all solutions hitherto employed. As will be noted all solutions were adjusted to a definite hydrogen ion concentration, cooled in ice and a stream of very finely divided oxygen passed in. The hydrogen ion concentration of the solution had not been tested after saturating with the gas. Therefore the decomposition of the sodium bicarbonate to sodium carbonate had been neglected. An examination of the solution after saturation showed that what was originally  $\text{P}_\text{H}$  7.4 was now nearly  $\text{P}_\text{H}$  7.8 an alkalinity far beyond anything which had been anticipated or desired. On titrating

20 c.c. of the solution with  $M/5$  acid sodium phosphate, it was found that it took 5 drops of this solution to raise the hydrogen ion concentration to 7.2.

*Experiment 11.* In order to obtain a solution of the requisite hydrogen ion concentration the following procedure was adopted.

A Tyrode's solution was made up omitting the bicarbonate. To 50 c.c. of this solution were added 50 c.c. of  $M/8$  glucose. The  $P_H$  of this mixture was 6.6. To 95 c.c. of the sugar Tyrode solution were added 0.5 c.c. of  $M/5$   $NaH_2PO_4$  and 1.8 c.c. of  $M/5$   $Na_2HPO_4$ . The  $P_H$  of this solution was 7.3 at  $18^\circ$ . A rapid stream of oxygen was passed in at  $0^\circ$  C. for 30 minutes. The  $P_H$  in the cold was 7.1; gentle warming to room temperature gave a  $P_H$  of 7.3-7.4. To the solution was now added 0.01 gram of dry sodium bicarbonate which was gently stirred in. The  $P_H$  of the solution when cold was 7.4. An emulsion of spermatozoa was now made with the cold solution and the tube placed in the thermos flask packed in ice. At the end of 72 hours the sperms were more active than I had ever seen them at this time. At 96 hours many of the cells were still active. When the preparation was examined at the end of 216 hours the cells were still in a state of motility. At this point the experiments were discontinued.

It would appear therefore that the combination of a properly balanced physiological saline solution with an isotonic glucose solution, a sufficiency of oxygen and a careful regulation of the hydrogen ion concentration it is quite possible to keep these cells in a condition in which motility can be restored on raising to body temperature, provided that in the interval they are kept at a temperature near  $0^\circ$  C. Each one of the factors mentioned above plays a definite part in promoting survival.

#### INSEMINATION EXPERIMENTS WITH SURVIVING SPERMATOOZOA.

Inasmuch as all my attempts to fecundate rabbits with spermatozoa which had been kept at a low temperature for varying lengths of time were apparently unsuccessful, they are not described in detail. Owing to Heape's statement that ovulation in the rabbit does not take place unless accompanied by coition, rabbits which from the appearance of the vulvae seemed to be in heat were covered by a vasectomised male. This animal had been prepared by Dr Marshall. If the doe accepted normally, a small quantity of sperm emulsion was injected into the vagina by means of a small glass syringe.

In certain cases there were signs of pregnancy, viz. swelling of the abdomen and "fleecing." The mammary glands appeared to be enlarged, but we were never able to satisfy ourselves that milk was secreted. In no case was a litter obtained. The conditions under which the rabbits were kept were not favourable for pregnancy. They were confined in open cages in a light room which was much frequented. This tends to the female eating her young. Iwanoff (p. 95) comments on the difficulty in making insemination experiments with these animals. Except that the period of gestation is short, they are not very suitable for this type of work. If they are to be used in future experiments, the following suggestions might be made.

Only does should be used which have given birth to litters of young a day or so previously. Ovulation takes place about this time and the ora uteri will be dilated so that the entrance of sperm will be facilitated. Advantage ought also to be taken of the method recently described by Dittler<sup>(24)</sup> for collecting rabbit semen. A doe is used in which the upper part of the uterus below the Fallopian tubes is tied off about 8.0 cm. above the vaginal orifice. By covering a rabbit prepared in this way, it is possible to obtain from 0.2 to more than 1.0 c.c. of semen. Furthermore, it would be of great advantage if the entrance of the sperm into the lumen of the uterus could be assured by dilating the vagina with a small speculum and passing the fluid through the uterine openings.

## PROTOCOLS.

Table I.

Soloid solution	P <sub>H</sub>	1 day	2 days	3 days	4 days	5 days	6 days
1. Control ... ..	6.1	Very active	—	0	—	Dead	—
2. Buffered ... ..	7.4	Very active	—	Majority active	—	Few active	—
3. Control + O <sub>2</sub> ...	6.6	—	Few moderately active	—	Dead	—	—
4. Buffered + O <sub>2</sub> ...	6.4	—	Many active	—	Few active	—	—
5. Control and glucose ... ..	—	—	6	—	0	—	0
6. Control and glucose buffered ...	—	—	Active	—	Majority active	—	Few active

Table II.

Solution buffered and glucose	P <sub>H</sub>	1 day	2 days	3 days	4 days	5 days	6 days	9 days
1. Locke's sol. (epididymis) ...	7.4	Very active	Little motility	Active	Active	—	?	—
2. Locke's sol. + O <sub>2</sub> (epididymis) ... ..	7.4	Very active	Quite active	Active	Greater than control	—	Active	—
3. Tyrode's sol. (suspension)...	7.5	Not very active	No action	Some action	—	Dead	—	—
4. Tyrode's sol. + O <sub>2</sub> (suspension) ... ..	7.5	Very highly active	Very active	Many active	—	Few moving	—	—
5. Tyrode's sol. (epididymis)...	7.5	—	—	—	—	Not very active	—	—
6. Tyrode's sol. (epididymis)...	7.5	—	—	—	—	Not very active	—	—
7. Tyrode's sol. ... ..	7.5	—	—	—	Few motile	—	—	—
8. Tyrode sol. + O <sub>2</sub> ... ..	7.5	—	—	—	Vigorous movement	—	—	—
9. Tyrode sol. + O <sub>2</sub> ... ..	—	—	Active	Active	—	—	—	—
10. Tyrode sol. and serum (1-10) + O <sub>2</sub> ... ..	—	—	—	Not so active	—	—	—	—
11. Tyrode sol. and serum (1-6) + O <sub>2</sub> ... ..	—	—	Not so active	—	—	—	—	—
12. Tyrode sol. + O <sub>2</sub> buffered with NaH <sub>2</sub> PO <sub>4</sub> ... ..	7.5	—	—	Quite active	No activity	—	—	—
13. Tyrode sol. + O <sub>2</sub> (glass distilled water) ... ..	—	—	—	—	—	—	—	—
14. Tyrode sol. + O <sub>2</sub> bicarbonate added after adjustment...	7.4	—	—	Most highly active	Active	—	—	Still motile



## SUMMARY.

By means of a suitable fluid and a low temperature, it has been possible to keep mammalian spermatozoa in a condition of potential motility for 9 days.

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A COMPARATIVE STUDY OF THE VALUE OF  
NITRATE OF SODA, LEGUMINOUS GREEN  
MANURES AND STABLE MANURE IN CYLINDER  
EXPERIMENTS, 1907-1919.

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(With Plates IV and V, and One Figure in Text.)

THE rapidly advancing cost of nitrogen in the form of commercial fertilizers and farm manures makes the study of the problem of securing this element through the use of leguminous crops, of unusual importance.

Furthermore, the decreasing supply of farm manure is causing the farmer to turn his attention more and more to green manure crops as a means of maintaining the supply of organic matter in the soil. With more definite and widespread information in regard to the use of lime, methods of inoculation, and the leguminous crops which are adapted to the varying conditions, the problem of growing these crops is becoming easier each year.

Such crops may be grown as a part of the regular rotation or they may be grown between the main rotation crops. For the Coastal Plain Section, where intensive farming is carried on, they are especially well adapted for the latter. Indeed the use of heavy applications of commercial fertilisers on these soils year after year without the use of farm manures or green manure crops, will result in unfavourable soil conditions, and crops will finally show indications of mal-nutrition.

From about 1890 until 1906 or 1907, the New Jersey Agricultural Experiment Station conducted many experiments to show the value of leguminous crops in building up light sandy soils, and in a carefully planned experiment conducted on a sandy soil in the southern part of the State, it was definitely shown that such soils can be built up in this way without the use of farm manures.

It seemed desirable, however, to have a more exact comparison of the value of nitrogen in nitrate of soda, green manure crops and farm manures on several different types of soil. In 1907, it was decided to conduct such an experiment, and in order that it might be carried out under uniform climatic conditions and under careful supervision, it was

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decided to bring a quantity of soil representing the different types to be studied, to the Experiment Station, and conduct the work on a small scale in cylinders set in the ground after the plan of the cylinder work on nitrogen availability which was started in 1898(6) and which is still in progress.

The cylinders used in this experiment were made of galvanised iron, 4 feet long and  $23\frac{1}{2}$  inches in diameter, being open at both ends like a piece of stove pipe. They have a surface area of 3 square feet which is  $\frac{1}{14,520}$  part of an acre. Before being placed in the ground they were thoroughly painted inside and out, and to date the majority of them appear to be still in good condition. The cylinders were set in the ground to within about 3 inches of the top. The local sub-soil was used for filling these cylinders to within about 10 inches of the top. On this sub-soil there was laid 200 pounds of the soil representing the respective types.

Such experiments do not give results that are directly comparable with field results, but the results secured from different crop and fertiliser treatments may be compared and are generally far more accurate than field results, for the reason that the crops are grown under much closer supervision and that less loss, due to unfavourable weather conditions, results. To illustrate, a rainy period just at harvest time may cause, and indeed has caused, a heavy loss of field-grown oats, whereas oats grown in the cylinders may be harvested between showers and taken into a building to be dried and carefully weighed without any loss. The same statement applies also to other grain crops and to hay and forage crops.

#### PLAN OF THE EXPERIMENT.

In order that the experiment might represent different sections of the State, eight types of soil were collected for the work as follows:

Windsor Sand.  
Norfolk Sand.  
Elsinboro fine sand.  
Quinton sandy loam.  
Collington sandy loam.  
Sassafras loam.  
Sassafras gravelly loam.  
Penn loam.

These soils have been described by Burke and Wilder(1) and the descriptions need not be repeated here. A later correlation by Marbut, Bennett, Lapham and Lapham(4) makes the following change:

Windsor sand to Norfolk coarse sand.

Norfolk sand to Sassafras sand.

Elsinboro fine sand to Sassafras fine sand.

Quinton sandy loam to Norfolk sandy loam.

It will be noted that the first seven of these are Coastal Plain soils and that the eighth is derived from the older triassic formation—sandstones and shales.

For carrying out this work a four-year rotation consisting of rye, corn, potatoes and oats was chosen. This rotation is not so widely used now as formerly, but it seems best to continue the work in accordance with the older plan.

A sufficient number of cylinders was provided so that the four crops could be grown on each soil type every year. The cylinders were run in duplicate with five different treatments for each crop. Since they are in duplicate, this gives ten cylinders to each crop (five treatments with two cylinders for each), and as there are four crops for each soil type, there are 40 cylinders for each of the eight types, or a total of 320 cylinders. The work has now been under way for 13 years and with eight types of soil each having four crops (with the exception of the rye in 1907), there are 32 crops each year or a total of 408 crops for the 13 years. Since there are ten cylinders for each crop, this gives 4080 single crops that have been harvested and analysed in the 13 years. In addition to this, 16 green manure crops have been grown each year, giving a total of 208 such crops.

#### FERTILISER TREATMENT.

The five duplicate treatments referred to above are as follows:

- (1) No fertiliser.
- (2) Minerals only (phosphoric acid and potash).
- (3) Minerals and nitrate of soda.
- (4) Minerals and leguminous green manure crops (as a source of nitrogen).
- (5) Minerals and stable manure.

All cylinders receive a liberal treatment of ground limestone once in five years. Nitrate of soda is applied at the rate of 5 grams per cylinder equivalent to 160 pounds per acre, acid phosphate at the rate of 20 grams per cylinder, and muriate of potash at the rate of 10 grams per cylinder. These materials are applied annually. Stable manure is applied at the rate of 15 tons per acre once in two years. The green manure nitrogen is supplied by seeding in two of the cylinders for each soil type, a legume

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crop immediately after harvesting the main crops (vetch and clover are seeded in the corn before the latter is harvested). These crops grow until near the time for planting the cylinders to the next crop, when they are spaded under, thus giving an effect equivalent to the ploughing under of a green manure crop in the field.

For example, after harvesting the rye, these cylinders may be seeded to soy beans and a mixture of vetch and clover. The soy beans will make a fair growth before frost and the vetch and clovers are left to grow until the following spring, when they are turned under for corn.

Soy beans after oats make a very good growth before time for seeding rye in the autumn.

A mixture of vetch and clovers is seeded in the corn, and soy beans and vetch and clovers after potatoes.

Since one of the objects of the experiment is to utilise the soil nitrogen to the fullest extent possible, corn is planted thick and harvested as forage rather than as ear corn. Potatoes are planted four hills to the cylinder and harvested in the usual way, both tubers and vines being weighed and analysed.

The oats are harvested at or near maturity, but in preparing the sample for analysis straw and grain are ground together. The rye is harvested at maturity and grain and straw saved separately. Nitrogen determinations are made in duplicate by the Kjeldahl method. The dry weight of the crop multiplied by the per cent. of nitrogen, gives the total nitrogen in the crop.

#### AVERAGE YIELD OF DRY MATTER.

The average yield of dry matter for each four cylinders (four year rotation in one year) for the five different treatments on the eight types of soil is shown in Table I (p. 328) and Fig. 1.

It is obvious that with 320 of these cylinders producing crops every year for 13 years, a large mass of figures would be accumulated. Therefore, in reporting yields of dry matter and nitrogen, the total yields for the four cylinders, representing the four crops of the rotation, are given, rather than the yield for each individual cylinder. The complete record of weights is, however, kept on file in the office of the Department.

Considering the yields for the different types of soil the weights for a given type remain fairly constant, or increase slightly, for the period 1907-1911 inclusive. For the green manure and stable manure series the maximum yield was reached in 1911; for the nitrate of soda and mineral series (check) in 1908.

Beginning with 1912 there is a general downward trend in the yields. There are exceptions to this, but without exception the *average* yields for the last eight years are distinctly lower than the averages for the first five years.

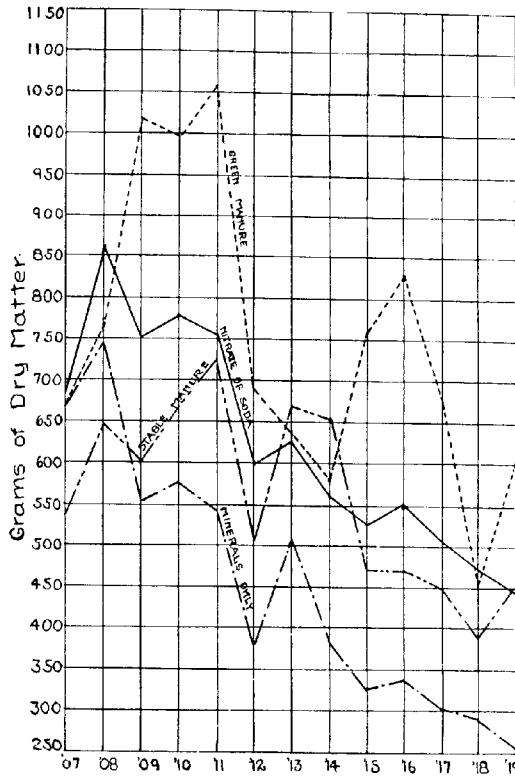


Fig. 1. Average yield of Dry Matter (grams per four cylinders, or one rotation) in a comparison of Nitrogen in the forms of nitrate of soda, legume green manures and stable manure, for the eight soils, from 1907 to 1919.

The green manure series forms the most notable exception. The yields for this series dropped notably in 1912, 1913, and 1914, and rose again in 1915 and 1916. It would appear that these fluctuations may be due to fluctuations of the green manure crops (which preceded the main crops) rather than to seasonal variations. Had they been due to

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the latter it would be expected that the other series would fall and rise with the green manure series. The crop data show that this was not the case. Notes made on the green manure crops of 1911, 1912 and 1913 state that the cow peas and soy beans in some of the cylinders showed signs of disease and did not make normal growth. Also in some cases the crimson clover, and vetch were largely winter killed. On the other hand the green manure crop of 1914, 1915 and 1916 (the green manure crop of 1914 would influence the *main* crop of 1915), were generally good. Again in 1917 the soy beans (the *green manure* crop for the 1918 *main* crop), in a number of the cylinders showed signs of disease. It would, therefore, appear that the low yields on the green manure series for certain years may be due, in part at least, to a partial failure of the green manure crops.

Table I.

*Average yield of dry matter for four cylinders, 1907–1919 inclusive.*

	Series I. No fertiliser	Series II. Minerals only	Series III. Minerals and nitrate of soda	Series IV. Minerals and green manure	Series V. Minerals and stable manure
	gm.	gm.	gm.	gm.	gm.
Windsor sand ... ..	345.2	450.4	656.1	781.2	481.0
Norfolk sand ... ..	255.6	265.9	452.8	541.4	386.6
Elsinboro fine sand ...	413.7	426.0	633.3	767.1	550.0
Quinton sandy loam ...	542.6	599.2	746.1	876.2	672.2
Collington sandy loam ...	385.4	466.9	625.0	771.0	548.9
Sassafras loam ... ..	363.1	461.6	641.0	755.7	538.2
Sassafras gravel loam ...	352.4	424.5	568.0	688.0	533.4
Penn loam ... ..	462.4	523.9	684.1	811.5	626.1
Average for 13 years ...	390.0	452.3	625.8	749.0	542.0
Average for first 7 years	486.0	560.9	723.1	832.2	607.9

Since the three series under consideration (nitrate of soda, green manure, and stable manure) received liberal applications of lime and mineral fertilisers, it would appear that the general decline in yields—especially in the case of nitrate of soda and stable manure series—must be attributed to a deficiency of available nitrogen. Or to state the matter in another way, since about 1911 nitrogen has been the limiting factor in all of these soils, and this has resulted in a gradually decreasing yield. This will be further considered in connection with the discussion on the nitrogen content of the soil.

A consideration of the average yields for the different treatments for the 13 years, shows that without exception they increase, for all types, from Series I to Series IV; that is, the no-fertiliser series stands lowest, the mineral series next, and the green manure series highest. The yields

on the nitrate of soda series usually run between the stable manure and green manure series.

When the yields for the different soil types are taken year by year, there are some exceptions to this statement, but when the general average is taken the results are as stated above.

These points are clearly brought out by the averages shown in Table I.

The lowest averages for the period are from Norfolk sand and the highest from Quinton sandy loam. The Penn loam, Collington sandy loam, and Sassafras loam stand next to the Quinton sandy loam in the order named.

These results are quite in harmony with general farm practice. The sandy loams are usually about as productive as the loams, even though the latter show a slightly higher content of plant food.

The explanation is to be found in the better physical condition of the sandy loams and consequently their superiority in the matter of aeration, drainage, earliness and ease of tillage.

The green manure crops have wrought wonders for the Windsor sand. From being one of the poorest of this series in 1907, it has risen to third place, yielding a general average of 781.2 grams, as compared with 811.5 grams for Penn loam, and 876.2 grams for Quinton sandy loam. The photographs in Plate IV show very distinctly their influence on the corn crop of 1916.

Calculated to the acre basis the average annual yield of dry matter for the green manure series, covering the eight types of soil, is 5939 pounds or nearly three tons per acre annually. The corresponding average for the check series is 3620 pounds. The difference—2319 pounds—represents the yield of dry matter that may fairly be credited to the influence of the green manure crops that each year preceded the main crop. This difference in yield in favour of the green manure series represents grain and potatoes as well as hay and forage, and therefore has a greater value than the same weight of dry matter in the form of hay or forage.

By the same method of calculation, it is found that the increase given by the nitrate of soda series over the check series is 1381 pounds and the corresponding increase for the stable manure series is only 578 pounds. However, in this connection, it should be remembered that the soils of the nitrate of soda series had been reduced in nitrogen content, by the end of the first five years, 0.01 per cent. more than those of the green manure and stable manure series.



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Certainly the increase made by the green manure series is a very creditable one and stands in strong contrast with the small increase shown by the stable manure series.

#### NITROGEN RETURNED IN THE CROP.

The nitrogen returns follow closely the yields of dry matter. For Windsor sand, Norfolk sand, Sassafras loam and Penn loam, the maximum average yield of nitrogen was reached in 1910 or 1911 and for the remaining type it was reached the first year—1907. From the point of maximum yield onward, the general trend has been downward, although in some years it rises slightly, but usually only to fall again the next year.

As in the case of the dry matter the average yield for the first five years surpasses, by a good margin, the average for the last eight years.

The Quinton sandy loam gave the highest yield of nitrogen through all the series, followed closely by the Penn loam. Without exception Norfolk sand gave the lowest average return.

From the standpoint of the different treatments the lowest averages for the eight types of soil were from Series I—the no-fertiliser series—and the next lowest from Series II—the check or mineral series. The returns from this series are in most cases only slightly higher than from Series I.

Series II, it will be noted, receives mineral fertilisers and lime in liberal amounts and should therefore be in a favourable condition for the fixation of atmospheric nitrogen by means of non-symbiotic organisms. However the yields from this series when compared with the yields from Series I, which are certainly not in a favourable condition for the development of non-symbiotic nitrogen-fixing organisms, do not indicate such fixation to any great extent.

The highest average yield for the eight types is found in Series IV—green manure nitrogen. The average yield for Series III—nitrate of soda—and for Series V—stable manure—run fairly close together though Series III is slightly ahead for five out of the eight types of soil. The averages referred to above are shown in Table II.

Since Series II differs from Series III, IV and V only in that it receives no nitrogen, it becomes the check series, and therefore, the difference between the yield of nitrogen on this series and any one of the nitrogen treated series represents the amount of nitrogen which the crop was able to win from fertiliser, manure, or green manure.

From Table II it is found that the 13-year average for Series II is 3.75 grams nitrogen and that the corresponding average for Series IV is 6.42 grams.

The difference—2.67 grams—represents the nitrogen won from green manure by the four cylinders in the rotation, or a cylinder average of 0.668 gram. This multiplied by 14,520, the fractional part of an acre which one cylinder represents, gives 9692 grams or 21.3 pounds of nitrogen per acre more than was recovered through crops on the check series. This is about what should be expected from the 2300 pounds of dry matter per acre that the legume series yielded in excess of the yield on the check series. Calculating in the same way it is found that the increase for Series III, over the check series, is about 10 pounds and for Series V about  $8\frac{1}{2}$  pounds per acre annually.

Table II.  
*Average annual yield of nitrogen for four cylinders, 1907-1919 inclusive.*

	Series I. No fertiliser	Series II. Minerals only	Series III. Minerals and nitrate of soda	Series IV. Minerals and green manure	Series V. Minerals and stable manure
	gm.	gm.	gm.	gm.	gm.
Windsor sand ... ..	2.85	3.34	5.13	6.33	4.06
Norfolk sand ... ..	2.22	2.23	3.76	4.52	3.59
Elsinboro fine sand ...	3.57	3.64	5.19	6.68	5.03
Quinton sandy loam ...	4.54	4.94	5.78	7.37	5.80
Collington sandy loam	3.43	4.03	4.91	6.68	4.92
Sassafras loam ... ..	3.27	3.88	5.06	6.56	4.78
Sassafras gravel loam	3.04	3.54	4.55	6.07	4.82
Penn loam ... ..	4.20	4.39	5.59	7.18	5.50
Average for 13 years ...	3.39	3.75	5.00	6.42	4.81
Average for first 7 years	4.20	4.73	5.91	7.32	5.43

From this it must not be concluded, however, that the legume crops won only 21 pounds of nitrogen from the air. Long time experiments(2) have shown that in the case of farm manures and other coarse organic matter only about one-third or less of the applied nitrogen is won back in the crop, and therefore in the case of green manures it is needless to expect a very much larger recovery. Hence the green manures must have won from the air something like 60 pounds or more of nitrogen per acre. The difference between this and the amount accounted for in the crop was lost in some way, either in drainage waters or as ammonia or gaseous nitrogen, into the air. It is exceedingly unfortunate that the loss should be so great.

#### INFLUENCE OF THE FERTILISER TREATMENT ON THE NITROGEN CONTENT OF THE SOIL.

It is of interest to learn whether the nitrogen supply of these soils can be better maintained under one of the systems of nitrogen treatment than another. In order to find this out it is necessary to know the

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nitrogen content of the soil at the beginning of the experiment and at the end of a given period, as for example, 5, 10 or 15 years, as well as the amount of nitrogen removed by crops during this time.

Table III shows the percentage of nitrogen present in the eight soils in their original condition (1907) and also the amount that was present at the end of five years, after the soils had been modified by cropping under the different systems of fertiliser treatment. (It is expected that analyses will be made again in 1921, the completion of 15 years.)

Table III. *Percentage of nitrogen in cylinder soils.*

Soil type	1912*					1907* Before treat- ment
	No fertiliser (lime only)	Check (lime and minerals)	Nitrate of soda (lime and minerals)	Green manure (lime and minerals)	Stable manure (lime and minerals)	
Windsor sand ...	·0410	·0423	·0402	·0522	·0527	·0653
Norfolk sand ...	·0273	·0265	·0274	·0349	·0372	·0319
Elsinboro fine sand ...	·0732	·0742	·0743	·0854	·0843	·0854
Quinton sandy loam ...	·0832	·0830	·0833	·0919	·0937	·1044
Collington sandy loam ...	·0741	·0770	·0742	·0861	·0863	·0894
Sassafras loam ...	·0830	·0837	·0849	·1000	·0934	·1111
Sassafras gravelly loam ...	·0566	·0570	·0522	·0658	·0676	·0961
Penn loam ...	·1242	·1258	·1293	·1369	·1359	·1722
Average ...	·0706	·0712	·0707	·08165	·0814	·0945

\* Gravel and other coarse material not removed. Whole sample ground, and determinations made on the sample thus prepared.

If we compare the 1912 figures individually with those of 1907, it is found that there has been a loss of nitrogen under all treatments for all types of soil except the green manure and stable manure series on Norfolk sand, and the green manure series on Elsinboro fine sand, which latter has just held even.

The averages for the green manure and stable manure series on the eight soils are slightly more than 0·01 per cent. lower than the original soil, while the averages for the other three series—I, II, III—are slightly more than 0·02 per cent. below the nitrogen content of the original soil.

It is thus shown that 15 tons of manure applied once in two years were not sufficient to maintain the nitrogen supply in seven out of eight types of soil under the cropping system adopted, and that the green manures did not maintain the supply in six out of eight of the soils. However, the fact that the green manure series gave larger crop returns than the stable manure, with no greater loss, or nitrogen exhaustion, must not be overlooked.

It is thus demonstrated that for the conditions under which this

work was carried out the green manure crops were more effective in the production of crops than 15 tons of manure applied once in two years, and that the nitrogen supply of the soil was maintained on an even balance with the stable manure series.

It must also be pointed out that while the soil of the nitrate of soda series contained no more nitrogen at the end of five years than the soil of the check series, it did, during this period, yield larger crops than the latter.

The average percentage of nitrogen in the soil is practically the same for the no fertiliser series, the check series, and the nitrate of soda series, but the latter produced larger crops and gave a larger return of nitrogen than either of the others.

In most cases the check, or mineral series, gave larger yields than the no fertiliser series, but in both of these series nitrogen has become the limiting factor to such an extent that all crops are very much reduced in yield.

It is not encouraging to find that the nitrogen supply of the soil has not been maintained by an average annual application of  $7\frac{1}{2}$  tons of manure per acre, nor by the green manure crops that have been grown on Series IV. Neither is it encouraging to find that the yields have been gradually falling for the last seven or eight years.

It must be remembered, however, that no crop residues other than roots and very short stubble have been returned to the soil, and that the extra care which has been given to this work has resulted in an average acre yield considerably above the yield that would be secured from the same crops had they been grown on a field scale. For example the green manure series has given a 13-year annual average equivalent to nearly three tons of dry matter per acre. The nitrogen thus removed together with the inevitable loss has meant a rather heavy drain on the total supply. It is well to remember in this connection that the loss of nitrogen has been intensified by the frequent stirring and the more or less thorough aeration of the cylinder soils. Therefore, it is not surprising to find that the crops are diminishing and that the soil is being depleted of its store of nitrogen and organic matter.

In the past hundreds of thousands of acres of our Coastal Plain Section have been thus treated, with the result that many farmers have been able to win from the soil only a bare existence or have entirely abandoned the farm for more profitable employment in industrial centres.

But, thanks to the introduction of better methods in general, and especially to a much wider use of lime compounds, which, in turn, has

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resulted in a much wider use of legumes for green manures, the farmer of to-day has much brighter prospects than the farmer of 20 years ago.

The encouraging feature of the work is to find that the green manures have maintained the nitrogen supply of the soil on an even balance with the soil which receives 15 tons of stable manure once in two years and in addition have yielded larger crops.

This work points the way to a more efficient utilisation, at an opportune time, of thousands of acres of sands, sandy loams and loams scattered throughout the Coastal Plain Section.

After all it is not necessary, and indeed hardly possible, to maintain a high nitrogen balance in such soils. Such a high balance can only be attained with very great nitrogen losses.

As bearing on this point the following quotation from Russell(5) is of interest.

Thus there is an upper as well as a lower limit to the nitrogen content of the soil, the actual values depending on the soil conditions. Between these limits the nitrogen content may be maintained at any desired level, high when the ground is left in grass and leguminous crops, low when the ground is continuously cultivated. Unfortunately, on our present knowledge it is impossible to maintain a high content of nitrogen on cultivated land except at a wasteful expenditure of nitrogenous manure.

The authors(3) have also shown that where heavy applications of manure are made annually, the loss of nitrogen is great, and that with this manure treatment, under a five-year rotation consisting of corn, oats, wheat and two years of grass, the nitrogen content of the soil was but slightly increased during a period of five years.

The secret of success lies rather in turning over to the crop each season sufficient available nitrogen to keep it in a normal growing condition, when other conditions are favourable.

It is entirely possible that this may be done on a percentage of soil nitrogen at a somewhat lower level than was found in the original soil. This level cannot, however, be indefinitely lowered without seriously menacing the crop yields and the permanent fertility of the land. The fact that this level of good yields and nitrogen content of soil can be maintained on Coastal Plain soils as well with green manures as with 15 tons of stable manure every two years, should furnish substantial encouragement to the farmers in this area who are finding it more and more difficult to obtain stable manure.

## SUMMARY.

1. The work presented in this paper covers a period of 13 years and gives the results secured, by means of cylinder experiments, in a comparative test of nitrogen in nitrate of soda, stable manure, and leguminous green manure crops, on eight types of soil, seven of which are Coastal Plain soils.

2. The work was carried out at the Experiment Station in galvanised iron cylinders similar to those used in the availability work which was started in 1898. 320 such cylinders were used, making it possible to run a four-year rotation on the eight types of soil with five different treatments for each type.

3. The five treatments are as follows: Series I—no fertiliser. Series II—minerals only (phosphoric acid and potash). Series III—minerals and leguminous green manure crops to supply nitrogen for the four main crops. Series IV—minerals and stable manure.

All cylinders receive liberal applications of ground limestone every five years.

4. The rotation consists of rye, corn, potatoes and oats, all grown every year on the eight soils.

Crops are harvested at or near maturity, dry weights recorded, and samples prepared and nitrogen determinations made in duplicate.

5. Under all of the treatments the largest crops were obtained during the first two or three years of the period. From the point of maximum yield which in most cases occurred within the first three years, there has been a gradual decline in yields, both of total dry matter and total nitrogen.

This decline, however, is not without some exceptions.

Without exception the average yields for the 13 years are less than the average for the first seven years.

6. Of the eight types of soil used Quinton sandy loam has consistently given the largest crops and the largest nitrogen return, followed closely by Penn loam. Norfolk sand has likewise consistently given the lowest returns.

Windsor sand has shown a remarkable response to the green manure treatment.

7. Of the five different treatments the green manure series has given the highest average returns on all the soils, although in a few cases scattered through the 13 years (and for the majority of the soil types during the first two years) the nitrate of soda series gave the largest

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yield. In a very few cases the yield on the stable manure series has exceeded that of the green manure series.

The average yield of the nitrate series stands between the green manure and stable manure series.

8. It has thus been shown that for a period of 13 years nitrogen supplied by leguminous green manure crops, grown between the main crops of the rotation, has been more effective in crop production than 15 tons of manure every two years, while at the same time the nitrogen content of the soil of the green manure series was maintained, for a period of five years at least, on a level with that of the stable manure series.

9. It has also been shown that the green manure series yielded larger crops than the nitrate ~~or~~ soda series (160 lb. of nitrate per acre annually), while at the same time the nitrogen content of the soils of the former was maintained at a higher level than that of the latter.

10. It is pointed out that it may be possible to maintain crop yields at a rather high level, even when the total nitrogen content of the soil is not kept at quite so high a level as was found in the original soil. Under such conditions, however, a constant turnover of readily available nitrogen is necessary.

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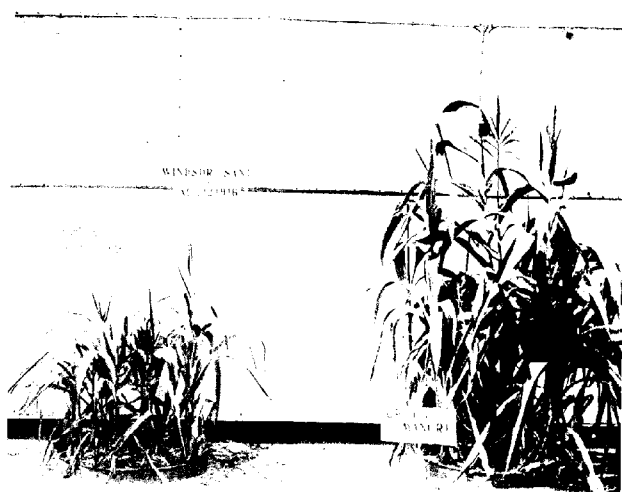


Fig. 1. Corn on Windsor sand. Green manure treatment compared with stable manure.



Fig. 2. Corn on Windsor sand. Green manure treatment compared with nitrate of soda.







Fig. 1. Soybeans and vetch used as a green manure crop on Windsor sand.  
(Photo, Oct. 12, 1915.)



Fig. 2. Soybeans and vetch used as a green manure crop on Windsor sand.  
(Photo, Sept. 26, 1916.)







## FURTHER OBSERVATIONS ON THE FACTORS CONTROLLING FERTILITY AND FOETAL ATROPHY.

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(With Plates VI and VII.)

### INTRODUCTION.

IN a previous paper<sup>(1)</sup> some of the factors which control fertility in domestic animals were discussed. These for purposes of clearness in investigation were divided into two groups: (1) The factors which control the number of eggs shed; and (2) The factors which control the number of eggs which develop to reach birth size. By this means the way in which the various factors which affect fertility could be determined, for example, it was shown that the effect of age on fertility, the literature of which has been reviewed by Harris<sup>(2)</sup>, is due to the number of eggs shed at each oestrus period. Young sows examined had on the average 14 corpora lutea in the ovaries while old sows had 20 corpora lutea.

It was observed that the fertility of domestic animals is very largely influenced by the second group. A large number of eggs fail to develop, some perish at an early stage, while others develop to a certain extent before they die and become mummified *in utero*. In these atrophic foetuses no evidence of bacterial action could be found and it would seem unlikely that the cause is bacterial in origin since normal and atrophic foetuses lie side by side in the uterus. In swine the *Bacillus abortus* has been found by Good and Smith<sup>(3)</sup> but it caused abortion of all the embryos and did not cause foetal atrophy.

Since the publication of the previous paper much other material has accumulated which bears on the cause of the foetal atrophy and the number of eggs shed at each oestrus period. The outbreak of war prevented earlier publication although much of the material quoted in this paper had been obtained just previous to its outbreak.

## METHODS AND MATERIAL.

The method of investigation has been to obtain the reproductive organs of pregnant animals and to count the number of corpora lutea in the ovaries and compare it with the number of embryos in the uterus. The number of corpora lutea was considered to correspond with the number of eggs shed at the time the animal was impregnated. In all the animals investigated there is very little chance that the corpora lutea are other than those of the oestrus at which gestation commenced, for it is extremely rare in animals that eggs are shed from the ovaries while corpora lutea of any size remain in them. In the ovaries of non-pregnant pigs corpora lutea of two ages can usually be discerned, but there is a distinct difference between them and it is almost impossible to confuse the two ovulations.

Rabbits do not usually ovulate unless coitus occurs so that there is very little chance of finding corpora lutea of the previous period in their ovaries. Rabbits may occasionally ovulate spontaneously as a result of one doe "jumping" another but this has been excluded by the fact that all the tame rabbits used have been kept in separate cages.

The number of corpora lutea could generally be determined by external inspection of the ovaries but occasionally when the numbers were large, and the corpora lutea closely packed, it was necessary to make a few incisions in the ovary.

The number of eggs shed is sometimes in excess of the number of corpora lutea in the ovaries as will be seen in the data given below, but its frequency is not sufficient to interfere seriously with any of the results. Corner<sup>(4)</sup> and others have shown that this is due to more than one egg developing in a Graafian follicle.

In all cases counts have been made with the fresh material as it was found much more difficult to count the corpora lutea in specimens which had been preserved.

The material has been obtained from the following sources:

*Pigs.* The pregnant uteri have been obtained from slaughter-houses and in the majority of cases the period of pregnancy (except by estimation from the size of the embryos) was unknown, as also were the previous conditions of feeding, management and fertility. In one or two cases through the kindness of Mr K. J. J. Mackenzie, Director of the University Farm, Cambridge, I have been able to obtain records of the previous history of a few sows which came from the University Farm.

*Sheep.* The pregnant uteri came from slaughter-houses and nothing

was known of the previous history of the animals. The period of pregnancy ranged from a few weeks to almost full term. All of the animals were killed during the months of February, March and April.

*Tame rabbits.* These were obtained in odd lots from dealers and were kept under uniform conditions in separate cages. They were mated and killed at known periods of pregnancy, the majority in the third week of gestation.

*Wild rabbits.* Some of the pregnant uteri were obtained from rabbits shot at Brampton, Norfolk, and others came from the rabbit warrens at Methwold, Norfolk. They were in all stages of the gestation period, the majority being about half term.

#### FREQUENCY OF FOETAL ATROPHY.

Foetal atrophy is quite general. The reason why it has not been observed more frequently is that as a rule absorption takes place either completely or the atrophic foetus is so reduced at birth that it passes unnoticed in the cleansings.

In those animals which have only one young at birth—mare and cow—the atrophy may pass unnoticed. It frequently happens that these animals “hold” to service for a month or two and then come “on heat” again; as no foetus has been aborted it is not usually definitely known whether the animals were pregnant or not.

Thus foetal atrophy while it only causes reduced fertility in those animals which have many young at birth may be a large factor in the sterile mare and cow problem. On the average of all the animals examined only about 70 per cent. of the eggs shed at oestrus become normal foetuses, the remainder either do not develop or become atrophic.

It is difficult to find atrophic foetuses in animals which have only one young at birth for, following the death of the young, the corpus luteum atrophies (when no more internal secretions come from the foetus). The atrophy of the corpus luteum frequently brings in its turn contractions of the uterus generally leading to expulsion of the foetus, although Weymeersch(5) has shown that this is not always the case and that sometimes embryos which perish at an early stage as a result of removal of corpora lutea are absorbed *in situ*.

The following summary gives an account of the mode of occurrence and frequency of atrophic foetuses that have been found in domestic animals:

*Cows.* One case occurred in March 1921 at the University Farm, Cambridge, and for this I am indebted to Mr K. J. J. Mackenzie. A cow



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which was served on December 28th, 1920, was noticed on March 18th, 1921, to have shed a lump of mucus and this when examined turned out to be the mucous plug of the cervix together with an atrophic foetus. Although the cow was at that time pregnant over 11 weeks the foetal membranes were of only about four weeks' development (as a comparison with foetal membranes of known stages showed). The foetus must have perished towards the end of January but the "abortion" did not take place until the middle of March. A blood sample from this cow was sent to be tested for contagious abortion immediately after the event but the result was negative. Several supposed cases of atrophy in cows are reported below but this is the only case in which the atrophic foetus has been found.

*Sheep.* The 80 pregnant animals examined had 116 corpora lutea in their ovaries and the uteri contained 101 normal fetuses and eight atrophic ones; there were seven eggs shed of which no trace could be found. It will be seen from these figures that the fertility of sheep is mainly influenced by those factors which control the number of eggs shed. Marshall(6) found that the normal percentage of ova discharged in the sheep at any single oestrus period was not apparently in excess of the usual percentage of births at the lambing season. The majority of atrophic fetuses which occur in sheep are found at an early stage. The atrophy seems generally to take the form, in the early stages, of an accumulation of fluid in the uterus with the degenerate foetus as a swollen gelatinous mass lying in it; when the atrophy occurs at a later stage however a cheesy mass is formed and the cotyledons in its immediate vicinity are usually small. None of the above-mentioned cases would have been observed in the cleansings of the ewe after parturition. During this season's (1921) lambing at the Cambridge University Farm there occurred one case of a ewe which gave birth to a normal lamb together with a small mummified one in its foetal membranes; the latter had evidently perished many weeks before it was born but at a later stage than any mentioned above.

*Pigs.* Table I gives the details of 22 pregnant sows which have been examined. It will be seen that the 22 sows contained 396 corpora lutea or an average of 18 per sow. Of these only 267 or 12.1 per sow had developed into normal piglings at the time of death and as the majority of sows were examined in the early stages of pregnancy there is no doubt that many more would have perished before the time of birth. There are no British figures available for the average size of the litter at birth but American figures range from 6 to 8 as the average size of the litter.

If the animals had been killed in the later stages of pregnancy it is probable that the 49 atrophic foetuses, an average of 2.2 per sow, which were found would have been increased. A total of 80 eggs or average of 3.7 per sow were unfertilized or had perished at a very early stage of development.

Table I. *Details of 22 pregnant sows. Number of corpora lutea and foetuses.*

No. of sow	Stage of pregnancy	No. of corpora lutea in ovaries	Number of			Remarks
			Normal foetuses	Atrophic foetuses	Missing ova	
I	About half term	24	13	10	1	—
II	" "	16	13	2	1	—
III	" "	17	13	1	3	—
IV	About 14 days	13	10	0	3	—
V	" 14 "	11	11	0	0	—
VI	" 1 month	24	21	0	3	—
VII	37 days	21	11	2	8	—
VIII	53 days	20	7	2	11	Previous litters: 11, 16 and 7
IX	32 days	17	17	0	0	Previous litters: 16 and 17
X	34 days	32	8	1	23	Previous litters: 10, 10 and 11
XI	About 80 days	14	7	3	4	—
XII	" 60 "	13	7	1	5	—
A 1	" half term	21	13	6	2	—
A 2	" 35 days	17	13	4	0	—
A 3	" 30 "	15	14	0	1	—
A 4	" 30 "	15	13	2	0	—
A 5	" 60 "	15	10	2	3	—
A 6	" 30 "	14	8	3	3	—
A 7	" 60 "	17	13	2	2	—
A 8	" 30 "	21	20	1	0	—
A 9	" 35 "	20	10	5	5	—
A 10	" 35 "	19	15	2	2	—
Total		396	267	49	80	—
Average		18	12.1	2.2	3.7	—
Per 100 eggs shed		67.4	12.4	20.2	—	—

From these figures it will be seen that in pigs the fertility is mainly influenced by those factors which control the number of eggs which develop.

The appearance of atrophic foetuses in the pig has already been described in a previous paper.

*Rabbits.* In the 56 tame rabbits examined the frequency of the occurrence of atrophic foetuses and missing ova is very similar to that found in pigs.

*Goats.* One case has been found in which there were two atrophic foetuses, both in the same foetal membranes; no normal foetuses were present.

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The occurrence of foetal atrophy has been recorded in many other species. Gynaecological literature is full of accounts of "blighted" fetuses or moles in man, and veterinary obstetrical works have frequent reference to their occurrence in mares, cows and sheep. Strahl and Henneberg<sup>(7)</sup> have described atrophic fetuses in the mole, hamster and ferret; Meyer<sup>(8)</sup> in guinea-pigs and rats; Fortuyn<sup>(9)</sup> in mice, and Kuntz<sup>(10)</sup> in cats and dogs.

#### SUPPOSED CASE IN COWS—EFFECT OF MALE PARENT.

During the winter of 1912-13 many of the cows at the University Farm, Cambridge, failed to "hold" to the bull, some going several weeks and others months after service before coming "into season" again. Veterinary examination showed that neither abortion nor granular vaginitis was the cause. Examination of the breeding records showed (see Table II) that all the cows which did not "hold" to service could be traced as being served by one bull—Seraphino. Table II shows that several cows "held" over three periods before coming "on heat" again—Scotch Kitty and Fair Princess going two months, Eaglethorpe Jocelyn over three months, Cowley Pleasure four months, Lovely four-and-a-half months and Dewdrop six months. It seems quite probable that in these cases fetuses were developed but became atrophic and were absorbed.

Stephenson<sup>(11)</sup> writing in 1885 said that "Some bulls apparently healthy, vigorous and good servers and bulls too that have been good stock getters appear to lose their procreative powers; cows that are served by them are seldom settled and if so often abort. There is a want of vitality varying in degree in the spermatozoa which prevents the ovum or foetus reaching maturity, thus causing abortion at different stages of gestation."

The semen of the bull (Seraphino) was examined in April 1913 and it showed (Pl. VI, fig. 1) only an occasional spermatozoa and these exhibited no sign of movement. On the other hand semen from the fertile bull (Prince Fame) was found to swarm with actively motile spermatozoa (Pl. VI, fig. 2). The semen of the bull Seraphino which was collected in November 1912 contained more sperms than when examined in April, although even at that time the numbers were very much below normal. Subsequent microscopic examination of the testes showed no pathological changes but only that the seminiferous tubules were in a state of physiological inactivity.

The reason why this bull should become infertile is problematical and various reasons have been suggested—excessive coitus, fatness, lack

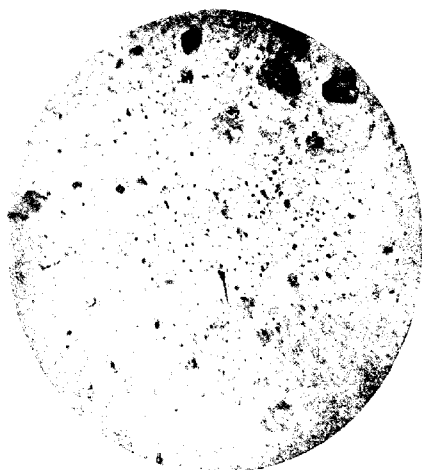


Fig. 1

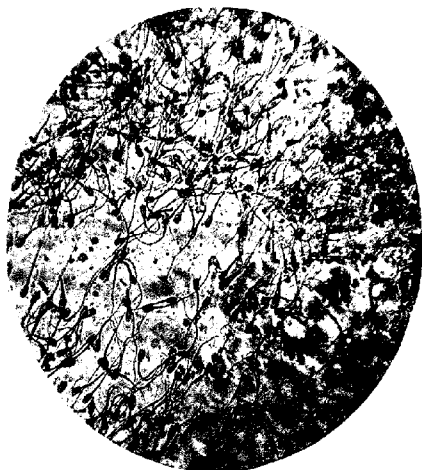


Fig. 2





Table III. *Effect of excessive coitus on fertility of rabbits. Sandy buck.*[illegible]

Experiment VI										Experiment VII									
No. of doe	Time of coitus May 6	No. of coitus that day	No. of young born	If killed			No. of doe	Time of coitus May 31	No. of coitus that day	No. of young born	If killed			No. of coitus that day	Time of coitus May 31	No. of doe	If killed		
				Normal embryos	Degenerate embryos	Missing ova					Normal embryos	Degenerate embryos	Missing ova				Normal embryos	Degenerate embryos	Missing ova
2	2.17 p.m.	1	6	—	—	—	1	1.41 p.m.	1	—	—	—	—	1	1.41 p.m.	1	—	—	—
	2.23	2						1.42	2					2	1.42		8	0	1
	2.32	3						1.44 $\frac{1}{2}$	3					3	1.44 $\frac{1}{2}$				
	2.45	4						1.53	4					4	1.53				
	2.50 $\frac{1}{2}$	5						2.7	5					5	2.7		8	0	1
	2.56 $\frac{1}{2}$	6						2.15	6					6	2.15				
	3.4	7						2.49	7					7	2.49				
	3.17 $\frac{1}{2}$	8						2.56	8					8	2.56				
	3.23 $\frac{1}{2}$	9						3.2 $\frac{1}{2}$	9					9	3.2 $\frac{1}{2}$				
	3.30	10						3.13	10					10	3.13				
	3.45	11						3.21 $\frac{1}{2}$	11					11	3.21 $\frac{1}{2}$				
	4.6	12						3.52	12					12	3.52				
	4.17	13						4.3	13					13	4.3				
	4.23	14						4.28	14					14	4.28				
	4.30 $\frac{1}{2}$	15						5.3	15					15	5.3				
	4.34	16																	
	4.41	17																	
	4.52	18																	
	5.6	19																	
	5.16	20																	
	5.27	21																	
	5.38 $\frac{1}{2}$	22																	
	5.45 $\frac{1}{2}$	23																	
	5.53 $\frac{1}{2}$	24																	
	6.14	25																	
	6.25 $\frac{1}{2}$	26																	
	6.33	27																	
	6.54	28																	
	7.10	29																	
	7.31 $\frac{1}{2}$	30																	
	7.51	31																	
	8.22	32																	
	8.50	33																	
	9.4	34																	
	9.12	35																	
	9.34	36																	
	9.46	37																	
	10.11 $\frac{1}{2}$	38																	
	10.27	39																	

All foetuses degenerate

3

6

Not pregnant—had not ovulated

Not pregnant



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of exercise, deficiency of vitamins in the food. The first has been investigated and the results which are given below show no effect; the others have not been investigated. Allen<sup>(12)</sup> found that atrophy of the seminiferous tubules of rats' testes were brought about by a diet deficient in water-soluble vitamins or by treatment with alcohol. The latter has also been observed by Arlitt and Wells<sup>(13)</sup> who found that it caused abnormal sperm formation. Stockard and Papanicolaou<sup>(14)</sup> found that male guinea-pigs treated with alcohol gave small litters and their offspring were frequently degenerate.

Hertwig<sup>(15)</sup> has shown that the sperms of tritons treated with thorium frequently penetrate the ovum which then develops but only to a limited extent; while Regaud and Dubreuil<sup>(16)</sup> found abnormalities of development to occur in rabbits after the sperms had been treated with Röntgen rays, although their results are open to criticism that abnormalities occur under natural conditions.

Whether or no the cows in this case actually had degenerate foetuses was not determined but it indicated that there was a possibility that fertilization by sperms which lacked vitality caused foetal atrophy. This is an explanation of a popular opinion that is held, viz. that some boars are "bad getters" and that litters from them are generally small.

The effect of different buck rabbits on the numbers in litters of does served by them has been tried in one or two cases and the results are shown in Table V. Although it would appear to indicate that the buck has an effect on the fertility of the does yet the numbers are too small to give absolutely definite results as it was not proved, by killing the does of each lot, that the number of eggs ovulated was the same in each case, although it is rendered probable by the fact that all the does had been fed and managed in the same way and all copulated on the same day. Buck R (Exp. XVI) is notably less fertile than buck A (Exp. XV) and the Lop (Exp. VIII) buck than the Dutch (Exp. I) one.

Marshall and Crosland<sup>(17)</sup> have shown that the percentage of foals "left" by different stallions varies from 27 to 75 per cent.

#### EFFECTS OF EXCESSIVE COITUS BY MALE PARENT.

It is a popular opinion that excessive coitus is a cause of reduced fertility and the regulations of the Ministry of Agriculture's Live Stock Scheme limit the number of females to be served by a male.

It was thought possible that if repeated copulations occurred that towards the end of the series unmaturing sperms might be ejected which

through incomplete fertilization would cause atrophy of the foetus or lead to the development of a weak embryo.

The effects of excessive coitus in the rabbit have been investigated and the results are given in the tables below. One buck was mated with

Table IV. *Latent period between successive copulations—Rabbits.*

Sandy buck; times in minutes and decimals of a minute.

Order of coitus	Experiment III			Experiment VI			Both experiments, Average latent period		
	Interval if same doe	Interval if fresh doe	Average of three copulations	Interval if same doe	Interval if fresh doe	Average of three copulations			
1-2	1	—	3.7	6	—	9.3	6.5		
2-3	—	3		9	—				
3-4	7	—		13	—				
4-5	—	5	6.0	—	5.5	6.3	6.1		
5-6	4.5	—		6	—				
6-7	—	8.5		7.5	—				
7-8	13	—	10.8	13.5	—	8.7	9.7		
8-9	—	7		—	6				
9-10	12.5	—		6.5	—				
10-11	—	0.5	4.3	15	—	15.3	9.8		
11-12	7	—		—	21				
12-13	—	5.5		11	—				
13-14	9.5	—	7.8	6	—	5.7	6.7		
14-15	—	6		7.5	—				
15-16	8	—		—	3.5				
16-17	—	6	7.2	7	—	10.7	8.9		
17-18	7	—		11	—				
18-19	—	8.5		—	14				
19-20	8.5	—	8.5	10	—	10.8	9.2		
20-21	Interval of 1 hr. 21 min. for lunch			11	—				
21-22	4	—	5.7	11.5	—	11.8	—		
22-23	—	5		—	7				
23-24	8	—		8	—				
24-25	—	4	6.0	—	20.5	13.4	—		
25-26	5.5	—		11.5	—				
26-27	—	8.5		7.5	—				
27-28	6	—	4.3	—	21	12.6	—		
28-29	—	3.5		16	—				
29-30	3.5	—		—	11.5				
30-31	Does "in season" difficult to find—13 min.			9.5	—	6.7	—		
31-32	5.5	—	10.0	Interval of 1 hr. 21 min. for supper					
32-33	—	10.5		7	—				
33-34	—	14		—	5				
34-35	10	—	7.0	8	—	19.8	—		
35-36	—	5		—	22				
36-37	6	—		12	—				
37-38	—	—	—	—	25.5	15.5	—		
38-39	—	—	—	15.5	—				
Average	Same doe 7.0	Fresh doe 6.3		Same doe 9.9	Fresh doe 13.5				

as many does as possible in a short time and the fertility of the does usually determined by the number of young produced at birth; in some

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cases however the does were killed during the course of pregnancy and the numbers of eggs shed and atrophic fetuses present determined.

The same buck was used in Experiments III to VII (see Table V). On March 25th (Exp. III) between 10.45 a.m. and 4.17 p.m. he copulated 37 times, 6 times on the following day and 9 times on the third day. On May 6th (Exp. VI) the same buck again copulated 39 times between 2.17 p.m. and 10.27 p.m. while on May 31st (Exp. VII) he copulated 15 times between 1.45 p.m. and 5.3 p.m. This buck died early in July, whether or no as a result of excessive sexual intercourse was not known. A glance at this Table (V) shows that except perhaps towards the end of the first day on March 25th there was very little reduction in the fertility of the rabbits served by him and this may be accounted for by the fact that does "on heat" were at that time difficult to obtain. Table III shows these experiments in detail with the exact time of coitus in each case. In passing it is interesting to observe the delay period between each coitus. The does were kept in separate cages, all in one house and there was no delay in putting a fresh doe in the buck's cage. Table IV, giving the result of these experiments, shows that at first there was a rather rapid increase in the length of the latent period between each coitus; which was followed by a period of more gradual increase and towards the end of the second series by a rather sharp rise. It also shows the shortening of the latent period due to an interval of rest and the ineffectiveness of introducing a fresh doe in reducing the length of the latent period.

In addition to this experiment a number of others of a similar kind have been made and Table V summarises the results of all these experiments which are negative as regards their effect on fertility.

In several of the experiments a determination of eggs shed and atrophic fetuses was made and the results are given in Table VI; these fail to show any increase in the number of degenerate fetuses and missing ova towards the end of a period of excessive coitus, except possibly towards the end of Experiment III, but at this time does which would copulate were difficult to obtain and the effect may be due to this. The high percentage of degenerate fetuses throughout this experiment may be due to the time of year or nutritional causes.

The general results of these experiments are not in agreement with those of Lloyd-Jones and Hays<sup>(18)</sup> who found that the percentage of pregnancies gradually decreased from 72 per cent. at the first service to 35 per cent. at the twentieth service. Table V shows no reduction in the percentage of doe rabbits breeding nor does it confirm their other con-



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clusion that there was a decrease in litter size between the fifteenth and twentieth services, litters of eight, nine and ten occurring between the twentieth and thirtieth services.

Table VI. *Effect of excessive coitus by male on occurrence of degenerate foetuses and missing ova—Rabbits.*

Sandy buck; figures bracketed denote the results from one doe.

Exp.	III—March 25			V—March 27			VI—May 6			VII—May 31			Average		
	Normal young	Atrophic foetuses	Missing ova	Normal young	Atrophic foetuses	Missing ova	Normal young	Atrophic foetuses	Missing ova	Normal young	Atrophic foetuses	Missing ova	Normal young	Atrophic foetuses	Missing ova
1	6	4	3	—	—	—	—	—	—	8	0	1	7	2	2
2	6	4	3	—	—	—	—	—	—	8	0	1	7	2	2
3	5	3	1	—	—	—	—	—	—	8	0	1	6.5	1.5	1
4	5	3	1	—	—	—	—	—	—	8	0	1	6.5	1.5	1
5	6	0	0	—	—	—	10	1	2	8	0	1	8	0.3	1
6	6	0	0	—	—	—	10	1	2	8	0	1	8	0.3	1
7	—	—	—	—	—	—	10	1	2	—	—	—	10	1	2
8	—	—	—	11	0	0	10	1	2	—	—	—	10.5	0.5	1
9	—	—	—	11	0	0	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	8	0	0	—	—	—	8	0	0
13	—	—	—	—	—	—	8	0	0	—	—	—	8	0	0
14	—	—	—	—	—	—	8	0	0	—	—	—	8	0	0
15	—	—	—	—	—	—	8	0	0	—	—	—	8	0	0
16	—	—	—	—	—	—	9	0	0	—	—	—	9	0	0
17	—	—	—	—	—	—	9	0	0	—	—	—	9	0	0
18	—	—	—	—	—	—	9	0	0	—	—	—	9	0	0
19	—	—	—	—	—	—	10	2	0	—	—	—	10	2	0
20	—	—	—	—	—	—	10	2	0	—	—	—	10	2	0
21	—	—	—	—	—	—	10	2	0	—	—	—	10	2	0
22	—	—	—	—	—	—	10	2	0	—	—	—	10	2	0
23	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
27	7	0	3	—	—	—	—	—	—	—	—	—	7	0	3
28	7	0	3	—	—	—	9	0	0	—	—	—	8	0	1.5
29	3	6	1	—	—	—	9	0	0	—	—	—	6	3	0.5
30	3	6	1	—	—	—	—	—	—	—	—	—	3	6	1
31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
34	1	0	11	—	—	—	—	—	—	—	—	—	1	0	11
35	1	0	11	—	—	—	—	—	—	—	—	—	1	0	11
36	1	8	2	—	—	—	—	—	—	—	—	—	1	8	2
37	1	8	2	—	—	—	—	—	—	—	—	—	1	8	2
38	—	—	—	—	—	—	8	0	0	—	—	—	8	0	0
39	—	—	—	—	—	—	8	0	0	—	—	—	8	0	0

The effect of the number of mares served in the season on the foaling percentages of stallions has been investigated from the returns of the Premium Heavy Horse Stallions under the Ministry of Agriculture's Live Stock Scheme during the years 1914-17 and here again the results (given

in Table VII) show no perceptible reduction in fertility as the number of mares per stallion increases.

Table VII. *Effect of number of mares served in the season on the foaling percentage of heavy horse stallions.*

Premium stallions of Ministry of Agriculture's Live Stock Scheme 1914-17.

Number of mares per stallion ... ..	10-20	20-30	30-40	40-50	50-60	60-70	70-80
Number of stallions from which results calculated	1	0	1	4	9	21	37
Foaling percentage ...	60.0	—	71.4	59.7	54.9	57.7	61.1
Number of mares per stallion ... ..	80-90	90-100	100-110	110-120	120-130	130-140	Over 140
Number of stallions from which results calculated	43	68	89	52	30	2	1
Foaling percentage ...	56.8	55.9	62.2	60.5	63.3	57.1	62.3

#### THE EFFECTS OF UTERINE NUTRITION.

It might be supposed that in animals such as the pig and rabbit where a large number of foetuses lie in the uterine horns that some would be crowded out through lack of space and consequently nourishment and so atrophy.

Table VIII. *Effect of the size of the litter on the weight of the young at birth—Rabbits.*

Number of young in the litter ... ..	1	2	3	4	5	6	7	8	9	10
24	144	177	210	246	368	355	451	527	434	
—	90	184	189	382	369	381	330	401	460	
—	145	188	191	264	326	376	518	464	447	
—	141	290	133	219	331	451	284	474	366	
—	97	105	124	219	373	248	250	328	—	
—	—	—	126	259	202	249	393	—	—	
—	—	—	153	222	319	267	370	—	—	
—	—	—	—	257	375	299	332	—	—	
Total weights of each litter at birth, gms.	—	—	—	—	312	296	360	361	—	—
—	—	—	—	—	290	373	345	370	—	—
—	—	—	—	—	303	312	236	414	—	—
—	—	—	—	—	268	258	381	296	—	—
—	—	—	—	—	204	256	397	357	—	—
—	—	—	—	—	264	—	396	290	—	—
—	—	—	—	—	222	—	424	337	—	—
—	—	—	—	—	177	—	263	351	—	—
—	—	—	—	—	173	—	401	—	—	—
—	—	—	—	—	—	—	278	—	—	—
Number of litters from which calculated ...	1	5	5	7	17	13	18	16	5	4
Average weight per litter, gms.	24.0	123.4	188.8	160.9	251.8	312.9	339.3	356.7	438.8	426.8
Average weight per rabbit, gms.	24.0	61.7	62.0	40.2	50.4	52.1	48.5	44.6	48.8	42.7

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The number of young present in the uterus affects the size to which they develop. Weighings have been made at birth of a number of litters of rabbits, and Table VIII shows that as the number of young produced at birth rises so does the total weight of the litter but the average size of each young rabbit decreases. This agrees with Minot's (19) results with guinea-pigs. King (20) found in rats that the young were dwarfed where the litters were large. Twin lambs weigh less than singles at birth and triplets weigh still less<sup>1</sup>. The foetal membranes in single lambs extend to both horns of the uterus and derive nourishment from them, whereas in twins the membranes are limited to the horn in which the foetus is lying. Measurements and weighings of the membranes and foetuses in pigs have been made and these show that the size of the foetus is roughly proportional to the extent of its membranes. As the embryos in the pig are orientated longitudinally along the uterine cornua the length of the membranes would be an indication of the area of uterus from which they are able to derive nourishment. In each of the sows II, III, A 3 and A 10 (for details of these see Table I) the membranes have been arranged (excluding the atrophic foetuses) in order of their length and then divided into three groups—longest, shortest and intermediate. The results for the four sows have been collated and the weights of the contained embryos are given in the following Table (IX).

Table IX. *The relation between the length of the foetal membranes and weight of the foetus—Pigs.*

#### Embryos and membranes of four sows.

The 18 embryos with the longest				foetal membranes, averaging 67.2 cm. weighed 31.9 gms.			
" 19	"	"	intermediate	"	"	"	58.5 cm. " 28.6 gms.
" 18	"	"	shortest	"	"	"	49.5 cm. " 27.5 gms.

As has been pointed out in a previous paper however (21) the size of the foetus and foetal membranes is not always dependent on the number in each uterine horn. Since the publication of this paper additional evidence has been accumulated which confirms this opinion. A large number of pregnant uteri of pigs, and tame and wild rabbits have been examined and the young contained in them weighed. Table X shows the average weights of the young from (A) the horn of the uterus which contained the most foetuses, (B) the horn of the uterus containing the smaller number of foetuses, and (C) cases in which the numbers in each horn were equal. It will be seen that both with pigs and tame rabbits the side with the largest number of embryos actually averaged the highest weight per embryo. However the side containing the smaller number of embryos

<sup>1</sup> It is hoped to publish shortly the data on which this statement is based.

more frequently averaged the higher weight per embryo. With wild rabbits the position is reversed. Where the number of young in each horn is equal there may be great disproportion in the average weight of the young on each side. It must be concluded that the size of the embryo is not dependent on the numbers lying in the uterine horn and that the size of the foetal membranes is due to a large extent to the vigour of the foetus from which it develops.

Table X. *Effect of number of embryos per uterine horn on their size.*

Two horns of the uterus of the same animal compared.

Animal	No. of litters	(A) Side with largest number of embryos		(B) Side with smallest number of embryos		Number of cases whose weight was:			(C) Equal number of embryos in each uterine horn			
		Average number of embryos	Average weight of embryos in gms.	Average number of embryos	Average weight of embryos in gms.	Heaviest in (A)	Heaviest in (B)	Equal	No. of litters	Average number of embryos	Average weight of embryos in gms.	
											Heaviest side	Lightest side
Pig	8	6.75	39.81	5.00	39.07	2	6	0	2	6.50	33.31	32.00
Tame rabbit	13	4.85	21.45	2.69	21.43	4	7	2	3	3.33	30.33	27.56
Wild rabbit	24	3.33	13.16	1.62	13.32	10	12	2	8	2.50	11.13	10.47

The weight of the young at birth is also influenced by the weight of the mother at parturition. This is shown for rabbits in Table XI and is no doubt partly due to genetic differences in size but is also probably due to the state of nutrition of the mother during pregnancy. The doe rabbits used were not alike genetically but this is counteracted by the fact that the bucks also differed so that there would be equal chances of a large

Table XI. *Effect of weight of mother on weight of the young at birth—Rabbits.*

Weight of doe, lbs.	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	10.0
Average weight of young at birth, gms.	35.0	46.0	50.8	41.3	43.4	53.7	54.4	52.5	61.7	55.3
	—	36.2	61.6	62.3	56.4	52.0	58.6	61.4	57.3	—
	—	34.6	49.2	42.7	44.7	42.1	54.4	—	—	—
	—	—	38.2	43.9	35.4	—	—	—	—	—
	—	—	43.0	—	37.6	—	—	—	—	—
	—	—	39.7	—	—	—	—	—	—	—
Number of litters from which calculated ...	1	3	6	4	5	3	3	2	2	1
Average weight per rabbit, gms.	...	35.0	38.9	47.1	47.6	43.5	49.3	55.8	56.9	61.0

doe being mated to a small buck and *vice versa*. King(22) found in rats that where the mothers were unwell during the period of gestation the young were dwarfed, and concludes that the body weight of a female influences the weight of her young chiefly because it depends on the two more important factors of age and physical condition(23). Zuntz(24) has shown that where pregnant female rats were starved the size of the



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young was reduced. Evvard<sup>(25)</sup> found that deficiencies in the rations of sows affected the size and vigour of their offspring and similar results have been obtained by Weaver<sup>(26)</sup>. Experiments at the Kansas Agricultural Experiment Station<sup>(27)</sup> have shown that rations deficient in ash caused a large number of dead pigs in litters, and Smith and Welch<sup>(28)</sup> found that deficiencies in the iodine content of the diet of pregnant sows caused weak piglings. Woll<sup>(29)</sup> attributed low birth weight in calves to a diet of lucerne only as compared with a mixed one, while Hart<sup>(30)</sup> found that the presence of bran instead of middlings in the rations of cows caused small size and low vitality of the calves.

The two conclusions reached above—(1) *That the average weight of the embryos decreases as the size of the litter increases*, and (2) *That the size of the foetus is roughly proportional to the extent of its membranes*, would appear at first sight to contradict the third—(3) *That the size of the embryo is not dependent on the number lying in the uterine horn*. However, this is not so, for the uterine horns have very great powers of expansion and they grow to accommodate the young, so that it is unlikely that the supply of nourishment is limited by this cause, nevertheless as the number of the young carried by the mother increases the supply will be limited. Thus twin lambs would have to share the nourishment available for a single one, which although usually ample may have occasional periods of deficiency affecting growth.

The importance of the above conclusions in relation to the occurrence of degenerate foetuses will be seen when the lengths of membranes of degenerate foetuses and the frequency of their occurrence in the horns of the uterus containing different numbers of young are compared.

A number of cases of degenerate foetuses in pigs have been found in which the foetal membranes still appeared to be living but in which the embryo itself had perished some time previously and was undergoing mummification (see Pl. VII, fig. 3).

This is in agreement with Strahl and Henneberg's<sup>(32)</sup> findings and also with Mall's<sup>(33a)</sup> and Myer's<sup>(33)</sup> conclusions formed from observations of a number of human cases, the chorion of which persisted for some time after the embryo itself had perished.

The first signs of approaching atrophy seem to occur in the blood vessels of the foetus and its membranes, these becoming congested and finally breaking down. Kaufmann<sup>(31)</sup> also found this to be the first sign of atrophy in salamanders.

In the case of one sow (A 10) the membrane lengths of normal foetuses ranged from 36 cm. to 72 cm. and one degenerate foetus was



FIG. 3





taken from the uterus with membranes 45 cm. long. As it occupied this extent of uterine "frontage" it seems unlikely that the cause of atrophy can be due to overcrowding in the uterus.

Kaufmann<sup>(31)</sup> with salamanders considers that the degeneration that occurs is not due to lack of nourishment as the relation of degenerate foetuses to the uterine wall is similar to the normal ones and moreover degenerate larvae contain quite a lot of yolk.

Table XII. *Correlation between the total number of foetuses per uterine horn and the number of degenerate foetuses—Pigs.*

Total number of foetuses per uterine horn	Number of horns which contained						Number of uterine horns from which calculated	Total number of foetuses	Total number of degenerate foetuses	% of foetuses which degenerated
	0	1	2	3	4	5				
12	—	1	—	—	—	—	1	2	24	29.2
11	—	—	1	1	—	2	—	4	44	34.1
10	—	1	—	—	—	—	—	1	10	10.0
9	2	—	—	1	—	—	—	3	27	11.1
8	1	2	1	—	—	—	—	4	32	12.5
7	5	2	1	—	—	—	—	8	56	7.1
6	1	2	3	1	—	—	—	7	42	26.2
5	—	2	1	—	—	—	—	3	15	26.7
4	4	1	—	—	—	—	—	5	20	5.0
3	1	—	—	—	—	—	—	1	3	0

Table XIII. *Correlation between the total number of foetuses per uterine horn and the number of degenerate foetuses—Rabbits.*

Total number of foetuses per uterine horn	Number of horns which contained					Number of uterine horns from which calculated	Total number of foetuses	Total number of degenerate foetuses	% of foetuses which degenerated
	0	1	2	3	4				
8	1	1	1	—	—	3	24	3	12.5
7	3	1	—	—	—	4	28	1	3.6
6	6	2	2	1	—	11	66	9	13.6
5	15	3	2	3	1	24	120	20	16.7
4	7	—	1	2	1	11	44	12	27.3
3	15	1	1	—	—	17	51	3	5.9
2	8	—	1	—	—	9	17	2	11.7
1	4	—	—	—	—	4	4	0	0

Degenerate foetuses too are not more frequent in the horns of the uterus which contain the largest number of young. Table XII for pigs and Table XIII for rabbits show that the percentage of atrophic foetuses does not rise as the number of foetuses per uterine horn increases. Cases in rabbits have been found where all the foetuses in one horn were atrophic.

Development abnormalities are known to occur and more especially at those times when the foetal membranes are being changed. Hill<sup>(34)</sup>

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found cleavage abnormalities in *Dasyurus*, and Huber<sup>(35)</sup> observed abnormalities in the development of mouse embryos before their attachment to the uterus, while Kirkham<sup>(36)</sup> has seen the same thing in the morula stage of the yellow mouse. In this connection it is interesting that in marsupials a reduction in the number of young occurs at the time of the change to the pouch; Hill and O'Donoghue<sup>(37)</sup> found that in *Dasyurus* large numbers perish at this stage. However foetal atrophy is not limited to these stages of change as degeneration may occur at almost all stages of development and not as Kirkham<sup>(36)</sup> supposes only at the periods of attachment and birth. Those that atrophy at the attachment stage would be classified in this paper as "missing ova" while those that degenerated after attachment would be shown as degenerate foetuses.

The general conclusion must be reached that the cause of atrophy is not limitation of nutrition by overcrowding in the uterus but by something innate in the foetus itself.

#### THE TIME OF YEAR.

The wild rabbit has a breeding season which lasts, depending to a certain extent on the weather, from about February to September, and this season in tame rabbits can be prolonged by controlling the temperature and food.

The effect of the time of year on the fertility of rabbits has been shown in Table XIV. In wild rabbits the number of eggs shed gradually increases with the rise of temperature from January to April (as Pearl<sup>(38)</sup> has found in the fowl) and then decreases; the numbers on which the decrease is based are however small.

Tame rabbits owing to conditions of housing have not such a marked curve of egg production but it is nevertheless quite appreciable, being highest in March and lowest in February and September.

These results differ from those of Marshall<sup>(39)</sup> in sheep as regards the breeding season, for he found that the generative activity of ewes tends to be greatest at the beginning of the sexual season; but the breeding season in sheep is different—September—and is associated with a falling and not a rising temperature.

The percentage of eggs which develop to normal young gradually falls, and the percentage of atrophic foetuses and missing ova rises, in wild rabbits during the commencement of the breeding season from January to April (see Table XIV) and this forms a possible explanation of Marshall's results in sheep. It may be due to the fact that egg production (induced by Heape's "generative ferments") is outstripping

ovarian nutrition but there is no direct evidence. As rabbits are often pregnant during lactation it may be that general and ovarian nutrition is decreased as the breeding season advances<sup>1</sup>.

In tame rabbits there is first an increase and afterwards a decrease in foetal atrophy as the season advances, corresponding with the rise and fall in the number of eggs produced. Koebner(40) however found that in tame rabbits atrophic foetuses were more frequent in August and September than in February to May but the numbers on which he bases

Table XIV. *The effect of the time of year on the number of eggs shed and the occurrence of atrophic foetuses.*

Tame Rabbits.					
Month in which pregnancy commenced	Number of pregnant rabbits from which calculated	Average number of eggs shed	Number of, per 100 eggs shed		
			Normal foetuses	Atrophic foetuses	Missing ova
January	—	—	—	—	—
February	5	8.4	61.9	14.3	23.8
March	8	12.0	41.7	21.9	36.4
April	—	—	—	—	—
May	26	10.6	72.7	6.9	20.4
June	2	11.0	81.8	0	18.2
July	—	—	—	—	—
August	—	—	—	—	—
September	9	9.1	82.9	1.2	15.9
October	6	10.0	65.0	5.0	30.0
Wild Rabbits.					
January	5	4.2	104.8*	0	0
February	4	4.5	94.4	0	5.6
March	45	5.8	89.7	2.3	8.0
April	13	6.7	73.5	4.6	21.9
May	—	—	—	—	—
June	1	3.0	100.0	0	0

\* 1 rabbit with one more embryo than corpora lutea.

his conclusions are small. Machens(41) who investigated the fertility of pigs from herd book records found that fecundity runs higher from September to March than in the warm periods of the year, from which it would appear that, as the fertility of pigs is largely controlled by the numbers which degenerate, the greatest numbers degenerate from March to September.

The general conclusion reached is that the seasonal changes affect the number of eggs shed from the ovary, and as these are increased so the percentage which develop normally is decreased and this is probably due to production outstripping nutrition.

<sup>1</sup> The effects of lactation on the fertility of rabbits are now being investigated.

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### EFFECTS OF DOMESTICATION.

The effects of domestication on fertility are very varied for while with most farm animals the number of young has been increased yet others kept in confinement such as monkeys, bears, elephants and chetahs, although apparently quite healthy, have breeding seasons and copulate, yet frequently either fail to produce young or the offspring are born ill-formed. No satisfactory explanation has been put forward of the effect of increased nourishment which follows domestication causing such divergent results. Possibly one should distinguish between the amount of nourishment and the suitability of the supply. It is notable that the effects of domestication are frequently extended not only to the production of ill-formed young but also to the limitation of the supply of milk<sup>(42)</sup> indicating an effect on the corpus luteum (see below) which controls both uterine and mammary nutrition. If this be so the foodstuffs used in domestication of certain animals may be lacking in the elements necessary for the proper formation of corpora lutea.

Table XV. *The numbers of eggs shed at the commencement of pregnancy, together with the percentage which after fertilization develop normally or become atrophic.*

Animal	Number of pregnancies from which calculated	Average number of eggs shed at commencement of pregnancy	% of eggs shed which became		
			Normal foetuses	Atrophic foetuses	Missing eggs
Pig ... ..	22	18.00	67.4	12.4	20.2
Sheep ... ..	80	1.45	87.1	6.9	6.0
Tame Rabbit ...	56	10.30	67.7	8.7	23.6
Wild Rabbit ...	68	5.74	87.2*	2.6	10.5

\* In one case one foetus in excess of the number of corpora lutea found.

The effects of domestication on the fertility of rabbits are shown in Table XV. It will be seen that the first effect is to increase the number of eggs shed at each period from on the average 5.74 in wild rabbits to 10.30 in tame rabbits—the numbers have been almost doubled. The average number of young at birth however—tame 7.3, wild 5.0—is only half as much again. Atrophic foetuses are much more frequent in tame than in wild rabbits. With the increase in the number of eggs produced has come increase in the proportion of missing and atrophic embryos.

Two possible explanations of the cause may be given—(1) That there is a competition for nourishment in the ovaries and that the effect of selection in domestic animals has been to increase the number of follicles which ripen at each period; frequently outstripping the supply of nourish-

ment available for them. While this explanation will account for the changes that have occurred in farm animals it will not explain the reduced fertility of bears, etc. kept in confinement.

Another more probable explanation is (2) That the effect of increased nourishment is to increase the number of eggs but that the nature of the nourishment being frequently unsuitable may be the cause of foetal atrophy; for example it is known that the tannin of acorns when eaten by fowls produces a dark coloured yolk in their eggs. This would explain the differing effects of domestication in different species, each varying with the nature of its food.

#### OVARIAN NUTRITION.

From the conclusions reached under the last two sections it would appear that ovarian nutrition is a very important factor in determining the number of atrophic fetuses. The occurrence of atrophic follicles in the ovaries of animals has long been known although the factors which cause them have been very little studied. Sandes<sup>(43)</sup> found that atrophy of the follicles was caused by the development of the corpus luteum in the ovary, whereas Robinson<sup>(44)</sup> found in the ferret many degenerate follicles out of the breeding season and the proportion of these to normal ones was about the same all the time. Loeh<sup>(45)</sup> discovered that in guinea-pigs underfeeding caused atresia of the follicles before they reached their normal size.

Table XV shows that the percentage of atrophic fetuses is high in pigs and tame rabbits where the number of eggs shed is high, and low in wild rabbits and sheep where the number of eggs shed is low.

The data obtained from pregnant sheep and pigs have been arranged to show the percentage of atrophic fetuses and eggs lost according to the number of eggs ripening per ovary. With sheep (Table XVI) the percentage of atrophic fetuses increases with the number of eggs per ovary shed. It will be seen that the percentage of atrophic fetuses is higher when two eggs are shed from one ovary than when one comes from each ovary. In cases where there was only one egg shed from both ovaries the percentage of normal embryos is too high, as cases in which degeneration occurred abortion or complete absorption would ensue and so would not be counted.

With pigs (Table XVII) the ovary with the largest number of corpora lutea has been taken as the standard for the animal and the individuals have been grouped according to this standard. It will be seen that the percentage of normal fetuses decreases, and the percentage of atrophic



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foetuses and eggs lost together (they should be considered together as some sows were in early and others late stages of pregnancy) increases, as the number of eggs shed per ovary increases.

Table XVI. *The relation between the number of eggs shed per ovary and the occurrence of degenerate foetuses—Sheep.*

Distribution of corpora lutea in the ovaries	Number of pregnant ewes from which calculated	% of eggs shed which became		
		Normal foetuses	Atrophic foetuses	Missing ova
1 in one ovary	45	100.0	0	0
1 in each ovary	18	86.1	5.6	8.3
2 in one ovary	16	71.9	15.6	12.5
2 in one ovary and 1 in the other	—	—	—	—
3 in one ovary	1	66.7	33.3	0

Table XVII. *The relation between the number of eggs shed per ovary and the occurrence of degenerate foetuses—Pigs.*

	Average number of corpora lutea—both ovaries	% of eggs shed which became		
		Normal foetuses	Atrophic foetuses	Missing ova
In 5 sows which had the largest number of eggs—average 17—shed from 1 ovary ...	22.6	53.98	14.16	31.86
In 5 sows which had a high average number of eggs—average 12—shed from 1 ovary	18.8	64.89	14.89	20.21
In 4 sows which had a low average number of eggs—average 10—shed from 1 ovary	16.7	71.64	16.42	11.94
In 5 sows which had the lowest number of eggs—average 8—shed from 1 ovary ...	14.8	74.33	10.81	14.86

The general conclusion reached is that the production of a large number of eggs per ovary, whether due to breeding, time of year, domestication or other causes, frequently outstrips the nutrition and leads to atrophy—either as follicles, newly fertilized ova, or as partially developed embryos. As has been pointed out above (Effects of domestication), the nutrition is more likely to be outstripped as regards quality than quantity. Robinson(44) states that follicles require the nourishment produced by an earlier set of follicles to enable them to grow. Hunter(46) found that when he removed only one ovary from a sow that she came "on heat" slightly earlier and gave almost as many piglings as the control animal. Carmichael and Marshall(46a) and also Bond(47) have shown too that in rabbits spayed on one side only, compensatory hypertrophy occurs on the other both in the interstitial cells and follicles.

## EFFECT OF YOHIMBINE ON FERTILITY.

It was thought possible that some drugs such as Yohimbine which has been found by Cramer and Marshall<sup>(48)</sup> to cause vaso-dilation of the generative tract might have an effect on fertility by increasing the blood supply and so the nutrition of the ovaries. Four experiments have been made in which Yohimbine in small doses, from 0.005 gm. to 0.02 gm. was given twice daily to rabbits for periods of three weeks before coitus. This in every case was without effect on fertility as will be seen from the following results of the experiments:

24 controls produced an average of 6.6 per litter and 29.2 per cent. of the does did not breed.

24 treated produced an average of 6.4 per litter and 41.6 per cent. of the does did not breed.

## EFFECT OF THE CORPUS LUTEUM.

Experimentally it has been found possible to produce atrophic foetuses by removal of all the corpora lutea from the ovaries in the early stages of pregnancy. Weymersch<sup>(49)</sup> removed the ovaries from rabbits in an early stage of pregnancy and found that the young were not aborted but absorbed *in utero*. In this case however all the foetuses became atrophic and no normals at all existed in the uterus. Fraenkel<sup>(50)</sup> has shown that the nutrition of the foetus is controlled by the corpus luteum. He removed by cautery the corpora lutea from the ovaries of pregnant rabbits and this was followed by foetal atrophy. The number of corpora lutea could be reduced to less than half the number of foetuses before any foetal atrophy was caused. In these experiments too all if any of the foetuses were caused to atrophy. It might be considered possible that when a large number of eggs are shed the supply of luteal tissue would be limited by the size of the ovary and so would not be sufficient to supply the needs of a large litter, but if so it would appear that all the embryos would be affected and not a few only.

## INBREEDING—LETHAL FACTORS.

Inbreeding has been shown to lead frequently to decreased vigour and fertility. Heape<sup>(51)</sup> showed that Dorset ewes served by rams of their own breed showed a greater tendency to barrenness than when served by Hampshire rams. Experiments at Wisconsin Agricultural Experiment Station<sup>(52)</sup> with fowls have shown that inbreeding reduced considerably the percentage of fertile eggs which hatched.

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Other observers have found no decrease in fertility due to inbreeding. King<sup>(53)</sup> found that selection for vigour in inbred rats is able to check the appearance of decreased fertility. The explanation has been put forward that when decreased fertility occurs it is due to the presence of lethal factors which were often intensified by inbreeding<sup>(54)</sup>.

Bauer<sup>(55)</sup> has shown that in *Antirrhinum* the death of some of the seedlings is due to the uniting gametes lacking the factor for chlorophyll, the young plants perishing at an early stage because no assimilation can take place. Boyd<sup>(56)</sup> found that in crossing bison and cattle an abnormal quantity of amniotic fluid was formed which was fatal in many cases. Lethal factors have been postulated to explain the non-occurrence of the expected Mendelian ratios in yellow mouse crosses, and since, of several other characters—waltzing mice, black-eyed mice (Little<sup>(57)</sup>). Subsequently uterine investigations were made and Kirkham<sup>(58)</sup> found in yellow  $\times$  yellow mice 37.8 per cent. of degenerate foetuses, whereas in albino  $\times$  albino mice he only found 2.3 per cent. degenerating. These results however are not based on the number of eggs shed but only of the foetuses found in the uterus. It will be noticed that the percentage degenerating in yellow mice is rather less than occurs in ordinary tame rabbits at certain times of the year—March (see Table XIV), and not very much more than occurs in tame rabbits as a whole (see Table XV).

Ibsen and Steigleder<sup>(59)</sup> certainly found a larger percentage of degenerate foetuses in yellow mouse crosses than in others, but unfortunately they give no counts of the number of corpora lutea found in the ovaries so there is no means of knowing what the proportion of "ova lost" was; the chances are that the proportions of these would be sufficient to account for the difference between the two types. Their results seem to show (if the total embryos is an indication of the total number of eggs shed) that the atrophy might well be attributed to ovarian nutrition, although the possibility still exists that yellow females tend to accumulate lipochrome pigment in the ovaries which affects the nutrition of the developing egg and which has been invoked to explain the sterility of fat cows (Marshall and Peel<sup>(60)</sup>).

It would appear that inbreeding, fatness and genetic differences associated with the yellow colour of mice are possible causes of foetal atrophy through their effect on ovarian nutrition. Although the possibility of lethal factors is evident yet it cannot be said that they have been proved to be the cause of foetal atrophy as no counts of corpora lutea or eggs shed have been made in these cases.

## CONCLUSIONS.

The pregnant uteri of ewes, sows, tame and wild rabbits have been examined and the number of foetuses counted and compared with the number of eggs shed from the ovaries when pregnancy commenced.

Fertility is limited in the higher animals by (1) the number of eggs shed at each heat period, and (2) the number of eggs shed which develop to normal young, *i.e.* by the occurrence of degenerate foetuses.

The fertility of domestic animals, especially the pig and rabbit, is largely controlled by the second group, whereas that of sheep and wild rabbits is limited rather by the first group.

Foetal atrophy occurring in cows is a cause of sterility. Defective sperm formation in the bull was thought to be the cause of sterility of this type which occurred in a herd of cows.

There is an indication in rabbits that males vary in fertility and that this affects the fertility of the females with which they mate, the data on which this conclusion is based however are not very extensive.

Excessive coitus in the male rabbit (up to 39 times in 8 hours) has no effect in reducing the fertility of the females with which he mates.

Heavy horse stallions serving 100 to 140 mares in the season are on the whole rather more than less fertile than those which serve 40-80 in the season.

Uterine nutrition has been studied to determine whether its insufficiency was the cause of foetal atrophy. The size of the young at birth depends on several factors: (1) the larger and better nourished the mother the larger the young; (2) the average weight of the embryo decreases as the size of the litter increases; (3) the size of the foetus is usually proportional to the extent of its membranes, but (4) in litters of rabbits and pigs the size of the embryo in any one animal is not dependent on the number lying in the uterine horn.

Foetal atrophy is not caused by overcrowding in the uterus because of (4) above, and also because the foetus itself atrophies before the foetal membranes on which its supply of food depends; moreover cases have been described by Kirkham in which the atrophy has occurred before the attachment to the uterine wall. The proportion of degenerate foetuses to total foetuses also is not greater in those horns of the uterus which contain large numbers as compared with those which contain small numbers of young.

In rabbits the number of eggs shed at the commencement of each pregnancy gradually increases with the rise of temperature from January

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to April and then decreases again. As the number of eggs shed increases so the percentage which develop normally decreases.

In rabbits the effect of domestication on fertility is to increase the number of eggs shed at each pregnancy from 5·7 in wild to 10·3 in tame. With the increase in the number of eggs produced however has come increase in the proportion of missing and atrophic fetuses—from 13 per cent. of the eggs shed in wild to 32 per cent. in tame.

The proportion of atrophic fetuses and missing ova is high in pigs and tame rabbits where the numbers of eggs shed are high, and low in sheep and wild rabbits where the numbers of eggs shed are low.

In sheep and pigs the proportion of atrophic fetuses increases with the number of eggs shed per ovary. In sheep it is higher where two eggs are shed from one ovary than where one comes from each ovary.

The production of a large number of eggs per ovary frequently outstrips the nutrition available for them and leads to atrophy—either as follicles, newly fertilized ova or as partially developed embryos.

The drug Yohimbine although it causes vaso-dilation of the generative tract in doe rabbits has no effect on fertility.

Experimentally Fraenkel found that foetal atrophy may be caused by removal of the corpora lutea but in this case all and not only a few of the embryos become atrophic.

It would appear that inbreeding, fatness, and genetic differences (associated with the yellow colour in mice) are possible causes of foetal atrophy through their effect on ovarian nutrition.

I am greatly indebted to Mr K. J. J. Mackenzie, Director of the University Farm, Cambridge, for many of the observations which he has allowed me to make on the animals there and to Mr Harvey, the farm manager, for his help in the collection of material.

My thanks are also due to the Ministry of Agriculture and Fisheries for the records of the Heavy Horse Breeding Societies which they put at my disposal, and lastly to Dr F. H. A. Marshall, F.R.S., whose advice I have continually sought throughout this investigation.

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## EXPLANATION OF PLATES VI AND VII

## PLATE VI

Fig. 1. Semen of sterile bull Seraphino, showing only occasional spermatozoa which exhibited no sign of movement.

Fig. 2. Semen of fertile bull Prince Fame, swarming with actively motile spermatozoa.

## PLATE VII

Fig. 3. Foetuses from the uterus of a sow (A1). IV and V—Normal foetuses with long foetal membranes. VIII—Normal foetus with short foetal membranes. VII—Foetus in very early stage of degeneration, blood-vessels congested. III and VI—Degenerate foetuses with part of foetal membranes still living (light portion). The foetus itself and part of the membranes are undergoing mummification (dark portion).

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# ON THE RELATIVE GROWTH AND DEVELOPMENT OF VARIOUS BREEDS AND CROSSES OF SHEEP.

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## INTRODUCTION.

Few records exist<sup>(1)</sup> as to the relative growth in weight of the different breeds of British sheep although quite a large number are available for foreign breeds and for British breeds kept under foreign conditions<sup>(2)</sup>, but the majority of these are for animals in a breeding as distinct from a fattened condition.

Very little is at present known of the way in which the proportions of the body in farm animals change as growth takes place. There exists no estimate as to the actual average amount of food produced by the different breeds on slaughter and the average proportions of consumable meat and offal in the dead body. Meek<sup>(3)</sup> has approached the problem from the point of view of external measurements of farm animals, but there has been no systematic attempt to work out the whole problem by weight as has recently been attempted in the case of the rat by Donaldson<sup>(4)</sup> whose work is the most complete account of the growth and development of any animal yet published.

The objects of the investigation recorded below were to supply data on these points and in addition to determine what were the various factors which influenced growth and development.

The results show not only the live weight of the various breeds of sheep and some of the conditions which affect it but also the carcase weight and proportion of the body on slaughter together with an account of some of the conditions which cause it to vary.

There still remains however another point which is of great importance in estimating the most economical breed for the production of meat, *i.e.* the proportion of bone, fat, and meat in the carcase. No measure is taken of this in the records published and the work could not be attempted on account of the expense and labour involved.



## MATERIAL.

The material and methods employed are similar to those used in a previous paper on the growth of cattle<sup>(5)</sup> and consist of a statistical treatment of the records of the Smithfield Club's Fat Stock Show from 1893 to 1913 inclusive, which I have been able to obtain through the kindness of Mr E. J. Powell, the Secretary of the Club.

It should be remembered when reading the paper that the results are of fat sheep only<sup>1</sup> and that all the classes that have been treated consist of wethers, *i.e.* castrated males. No sex differences are therefore shown but these are quite large in sheep as collected material which it is hoped to publish shortly will show.

From a scientific point of view the conclusions arrived at are lessened in value by the fact that the qualities of growth and ability to fatten are combined, but from a practical standpoint the conclusions are correct as these processes will generally go on concurrently.

Two series of competitions for sheep exist at the Smithfield Show: (1) the Live Classes and (2) the Carcase Classes, and in both these series only wethers, *i.e.* castrated males, are exhibited. In the earlier years of the Show ewes were also exhibited but these have been excluded from the calculations made in this paper.

In the first series—Live Classes—a record is kept only of the gross weight and age of the animal. The series is divided into groups for each breed and type of cross bred and these groups are subdivided into classes according to age.

In the second series—Carcase Classes—in addition to the records of age and gross weight, details of the weight of the carcase, fat, skin etc. after slaughter are also kept. This series is divided only into groups for pure breds and cross breds of different types and subdivided into classes for the different ages.

The entries for this series are very small compared with the number of exhibits in the live classes especially in view of the fact that the carcase test is the ultimate object for which the sheep were bred.

The weighing and recording of weights at the Show have been done very accurately for the period under review. I am indebted to Mr F. Lloyd, of Messrs Simpson and Lloyd, who has kindly supplied me with the details of the methods used which have been the same for the whole period of 21 years.

<sup>1</sup> It is to be regretted that other Agricultural Shows keep no records of the weights of breeding animals exhibited.

## METHODS.

The details given in the records of the Show have been treated statistically so as to give information on the points it was desired to investigate.

In order to avoid confusion when discussing the results of the investigation below, the following account gives the methods by which the tables in the text have been compiled:

Weights throughout have been calculated in lbs. and decimals of a lb.; ages have been calculated in months and weeks.

*Table I* has been compiled from the records of the Show direct. The Classes are open for "pens of three sheep" so that in compiling this table the weight as shown in the records has been divided by three to give the weight of each individual. The divisions "Under 12 months old" and "Over 12 months old" correspond with the divisions into classes at the Show. The material has been grouped into three seven-year periods: Period I from 1893 to 1899 both inclusive; Period II from 1900 to 1906 both inclusive; Period III from 1907 to 1913 both inclusive. The averages for sheep exhibited in each period have been shown separately and at the end the average for the whole 21 years has been given.

The number of animals from which the average has been calculated is stated as a measure of the accuracy of the average. Averages calculated from less than 40 individuals are shown in italic type.

*Table II* has been calculated from the average columns of *Table I* to eliminate the differences due to the various ages at which breeds were exhibited. These calculations have been made by estimating the rate of growth per week in each case (the weight at birth being taken as zero) and then adding or subtracting the number of weeks growth required to complete 9 or 21 months. The weekly rate of growth has been estimated in the following way: between birth and 9 months:

$$\frac{\text{Average weight at under 12 months}}{\text{Average age at under 12 months}},$$

and between 9 and 21 months:

$$\frac{\text{Average weight at over 12 months} - \text{average weight at under 12 months}}{\text{Average age at over 12 months} - \text{average age at under 12 months}}.$$

In cases where no sheep were shown at 9 months the correction has been estimated from other breeds of approximately the same weight.

The weight at birth has been neglected as no reliable data exist as to the weight at birth of the various breeds and it was considered better to neglect it rather than subtract a round figure for all breeds alike.

Averages calculated from less than 40 individuals are shown in italic type. The probable error of the mean has been calculated in several cases and these are shown in Table A.

Table A. *Live weight of sheep—probable error of mean*  
(pen of three), in lbs.

BREED	9 MONTHS	21 MONTHS
Leicester	1.087	1.415
Blackface	1.253	1.445
Cheviot	1.353	1.768
Southdown	0.430	0.671
Hampshire	0.558	1.080
Suffolk	1.080	1.294
Southdown × Hampshire	1.443	—

*Table III* has been compiled from the records of the Carcase Competitions direct. The Carcase Competitions were instituted in 1896 and the averages given are from that year to 1913 inclusive. The divisions "Under 12 months old" and "Over 12 months old" correspond with classes at the Show. In each case the number of sheep from which the average has been calculated is given as a guide to the accuracy of the average. Where the average has been calculated from less than 20 individuals it is shown in italic type. The following with the exception of the first is an explanation of the parts into which the animal is divided after slaughter:

*Live weight.* Is the live weight of the animal as sent to the Show. For some years (1896–1902) "Live weight" and "Starved Live weight" were both recorded. Only the former however has been utilised in this paper. The effect of starving has a considerable, although as yet not accurately determined, influence on the carcase percentage of the animal, for at 9 months old the food contained in the stomachs and alimentary canal is on the average about 7 per cent. of the live weight, but the whole of this cannot be eliminated by starvation.

*Carcase weight.* Consists of the dressed carcase of the animal after it has been killed and the parts mentioned below removed. An allowance is made for the weight of the head and the feet on the carcase—6 lbs. for carcases weighing under 64 lbs., 7 lbs. for carcases weighing between 64 and 80 lbs., and 8 lbs. for carcases weighing over 80 lbs.—and this has been deducted from the weight of the carcase by the officials at the Show.

All carcase weights published in this paper are weights without head or feet, these being included in the column "Unaccounted for."

*Fat.* Consists of the combined caul (omentum) and gut (mesenteric) fat. From weighings of Suffolk sheep of Smithfield standard made by the writer the proportion of these is as follows:

	Caul fat	Gut fat
9 months	3.7	1.6 % of body weight
21 „	5.9	1.9 „ „

*Pluck.* Consists of the heart, lungs, trachea, liver and part of the oesophagus and diaphragm with attached fat and one thymus gland.

*Skin.* Consists of the skin and wool.

*Unaccounted for.* Consists of all those parts which were not weighed—blood, bladder, stomachs and intestines with contents—as well as the allowance for head and feet together with the loss of water by evaporation that occurs on slaughter. From weighings that have been made by the writer the percentage of these organs in Suffolk sheep of Smithfield standard is as follows:

Nine months old: blood, 3.7 %; stomachs, 2.7 % and contents 5.2 %; intestines, 2.1 % and contents 1.7 %; head and feet 4.7 % making a total of 20.1 % which is fairly near the total “Unaccounted for” shown in Table V.

*Table IV* has been compiled from *Table III* in the same way that *Table II* has been compiled from *Table I* by adding or subtracting the number of weeks growth required to complete 9 or 21 months. At 21 months old the correction is based on the difference between the weight at “Under one year old” and “Over one year old.” In cases where no sheep were shown at 9 months old the correction has been estimated from other breeds of the same weight.

Averages calculated from less than 20 individuals are shown in italic type.

*Table V* has been calculated from *Table IV* by showing the carcass and various organs as a percentage of the live weight. At the end of this table an average has been compiled of all pure bred sheep exhibited. This average is the mean of all the sheep exhibited and is not the average of all the breeds.

Averages calculated from less than 20 individuals are shown in italic type. The probable errors of the means have been calculated in a few cases and are shown in *Table B*.

*Table VI* has been calculated from *Table IV* in the same way that *Table VII* has been compiled from *Table II*, the weight of each component at 9 months being expressed as a percentage of the weight of that component at 21 months.

Table VII has been calculated from Table II by expressing the weight of each breed at 9 months as a percentage of that at 21 months. The numbers from which the results have been calculated are given as a guide to their reliability. The probable error has been calculated from the formula

$$\frac{(\text{P.E. of } P)^2}{P^2} = \frac{(\text{P.E. of } M_2)^2}{M_2^2} + \frac{(\text{P.E. of } M_1)^2}{M_1^2},$$

where  $P$  = percentage of  $\frac{M_2}{M_1}$ , and refers to the average of a pen of three (cf. above). In the case of Hampshires and Southdowns it is  $\pm 0.0326$  and  $\pm 0.0311$  per cent. respectively.

Table B. *Proportion of organs in sheep—probable error of mean in per cent. of live weight.*

BREED	9 MONTHS					21 MONTHS				
	Carcass	Fat	Pluck	Skin	Unac- counted for	Carcass	Fat	Pluck	Skin	Unac- counted for
Cheviot	.28	.11	.04	.13	.35	.30	.09	.03	.13	.31
Hampshire	.22	.09	.03	.13	.24	.53	.16	.05	.12	.50
Suffolk	.18	.07	.03	.09	.20	.29	.11	.03	.09	.31
Southdown	.22	.10	.04	.14	.23	.38	.11	.04	.09	.40

Table VIII has been compiled from the records of the Show in the same way as Table I except that the crosses in this table have not been grouped by periods. In column 1 the male parent is shown first in each case. Averages calculated from less than 20 individuals are shown in italic type.

Table IX has been calculated from Table VIII in the same way that Table II has been prepared from Table I. For purposes of comparison the mean weights between the two pure breeds at each age are shown in the last column. Averages calculated from less than 20 individuals are shown in italic type.

Table X has been compiled from Table IX in the same way that Table VII has been prepared from Table II. Results calculated from less than 20 individuals on either side are shown in italic type.

Table XI has been compiled from the records of the Show in the same way as Table III. The male parent of the cross is shown first in column 1. Results calculated from less than 10 individuals are shown in italic type.

Table XII has been prepared from Table XI in the same way that Table IV has been compiled from Table III. Results calculated from less than 10 individuals are shown in italic type.

*Table XIII* has been compiled from *Table XII* in the same way that *Table V* has been calculated from *Table IV*. Results calculated from less than 10 individuals are shown in italic type.

*Table XIV* has been prepared from the records of the Show by first correcting the weight of each animal for age (see *Table II*). The standard deviation has been calculated from these corrected weights according to the formula  $\sigma + \sqrt{\frac{\sum D^2}{n}}$ , and from this the coefficient of variability

calculated according to the formula  $\frac{\sigma}{M} \times 100$ . The number of animals on which the calculation is based being given in each case. The sheep have been shown in pens of three so that the figures shown in the table are deviations and coefficients of variability on the average of pens of three.

*Table XV* has been calculated in the same way as the previous table but the animals have been grouped according to the period (see *Table I*) in which they were exhibited. The mean used in the calculations is the mean of the whole 21 years.

*Table XVI* has been calculated from the records of the Carcase Competition by converting the weights of each individual, as given in the records of the Show, to percentages of live weights; the coefficient of variability being calculated in the same way as in *Table XIV*.

*Table XVII* has been compiled from the records of the Carcase Competition by converting the weights of the carcase and various organs of each individual to percentages of the live weight. Individuals of the four breeds—Southdown, Suffolk, Hampshire and Cheviot—have been treated in this way. The animals of each of these breeds were then arranged in order of proportion of the organ selected for correlation and divided as equally as possible into four classes. The numbers of each breed which fell into the various classes were as follows:

BREED	9 MONTHS				21 MONTHS			
	Highest	High	Low	Lowest	Highest	High	Low.	Lowest
		average	average			average	average	
Southdown	20	20	20	20	15	14	14	14
Suffolk	39	38	38	39	16	15	15	15
Hampshire	21	21	21	21	8	9	9	9
Cheviot	16	17	17	17	19	19	19	19

As the numbers for each breed were too small to give by themselves a reliable result the whole have been combined by averaging the groups in each breed; thus the figures given in this table are the average of four breeds.

*Table XVIII* has been prepared from *Table I* by correcting for age (see *Table II*) in each of the averages of the seven-year periods. The mean of the average of all the breeds which is given at the bottom of the table is calculated from the breed averages and no account has been taken of the number of individuals.

*Table XIX.* The records on which the figures for 1840-42 are based are those published in the *Farmers' Magazine* for those years. The 1893-1913 "live weights" have been calculated in each case from the live weight by using the percentage which the breed averaged in the Carcase Classes for those years.

*Table XX* has been compiled from *Table III* by correcting for age (see *Table IV*) in each seven-year period of four of the best represented breeds. These results have been converted to percentages of live weight. The averages of all the individuals of the four breeds exhibited in each period are given at the end of the table.

*Table XXI* has been prepared by calculating the difference between the average weight of all the sheep of a breed exhibited in one year and the breed average for the whole 21 years. Five breeds—Leicester, Southdown, Suffolk, Hampshire and Cheviot—have been treated in this way and the plus and minus quantities for all the breeds in each year have been totalled and are shown in the table. The fourth column gives the difference between the yearly average rainfall for England and Wales<sup>(6)</sup> and the average for the period 1893-1913. In the same way the fifth and sixth columns give the differences for the turnip crop and the combined permanent and temporary hay crops<sup>(7)</sup> respectively. Wethers of 21 months old are placed under the year in which they were born as it was considered that conditions during the first year would have more effect on growth than those occurring in the second year.

#### RESULTS.

The results obtained by this investigation are considered below in sections under various headings. These—breed, age, early maturity, crossbreeding, individual variation, selection, correlation and season—comprise the different factors which it was considered might have an effect on growth and development.

In general the results obtained from the Live Classes for actual gross weight have been dealt with first and afterwards the proportional development as shown by the Carcase Classes has been considered.

**Breed.** *Table I* shows the average weights and ages of the various breeds as calculated direct from the "Live class" records of the Show.





The average age is a good guide as to the time of lambing of each breed, and it will be noticed that there is a general tendency for it to become earlier, but owing to these differences the figures for weight will not be strictly comparable. Table II has therefore been prepared in which all the breeds have been calculated to common ages—9 months and 21 months—these being selected because the majority of the animals were of these ages.

Table II. *Estimated comparative weights and rates of growth of different breeds of sheep.*

Weights in lbs. and decimals of a lb. Calculated from average columns of Table I by correcting for age differences.

BREED	9 MONTHS OLD			21 MONTHS OLD		
	No. of sheep	Weight	Weekly rate of growth between birth and 9 months	No. of sheep	Weight	Weekly rate of growth between 9 and 21 months
			9 months			21 months
Lincoln	201	198.0	5.50	222	326.5	2.55
Cotswold	99	188.0	5.22	57	296.2	2.25
South Devon	48	196.2	5.45	21	281.4	1.62
Suffolk	366	187.6	5.21	219	280.6	1.79
Leicester	246	172.4	4.79	267	280.0	2.35
Oxford Down	303	174.5	4.85	255	275.5	1.85
Hampshire Down	744	176.5	4.90	429	273.3	1.68
Kent	357	170.1	4.73	361	270.7	2.25
Wensleydale	—	—	—	3	266.0	(2.30)*
Dorset Horn	183	158.4	4.40	78	260.6	1.60
Dorset Down	57	156.5	4.34	—	—	—
Devon Longwool	156	179.0	4.97	96	255.6	1.59
Border Leicester	27	157.8	4.38	30	246.8	1.95
Shropshire	273	158.0	4.39	339	244.2	1.79
Kerry Hill	—	—	—	87	221.4	(1.40)*
Cheviot	174	161.5	4.49	306	216.9	1.47
Blackface	165	148.5	4.12	309	210.2	1.55
Ryeland	3	136.6	3.79	117	203.0	1.48
Southdown	963	137.4	3.82	660	202.4	1.22
Welsh Mountain	—	—	—	144	135.0	(1.15)*
Exmoor Horn	—	—	—	58	133.3	(1.15)*

\* Estimated.

The large number of individuals from which the average has been calculated in most cases ensures that the results are fairly reliable.

In Table II the breeds have been arranged in order of their weight at 21 months old, the mature size of the breed; the differences in the relative order at 9 months old are due to variations in the rate of maturity and will be discussed below.

The Lincoln is by far the heaviest breed, the Cotswold coming second, being some 30 lbs. lighter. These are followed by a number of breeds which only differ among themselves from heaviest to lightest by about

10 lbs., the heaviest being some 15 lbs. lighter than the Cotswold—South Devon, Suffolk, Leicester, Oxford Down, Hampshire Down and Kent in the order named. The Wensleydale, Dorset Horn and Devon Longwool are a few lbs. lighter and the Border Leicester and Shropshire some 10 lbs. lighter still. There is then a fairly big drop of 20 lbs. before the next group—Kerry Hill, Cheviot, and Blackface—closely followed by the Ryeland and Southdown. There is then a very large drop of 70 lbs. between these and the two smallest breeds—Welsh Mountain and Exmoor Horn.

It will be noticed that at 9 months old the following breeds take a relatively higher place in the table. The South Devon comes second and the Devon Longwool takes a place next the Suffolk and is closely followed by the Hampshire Down and Oxford Down, these both displacing the Leicester. The Cheviot also advances above the Dorset Horn. The significance of these changes will be discussed below under "Early Maturity."

The results of the Carcase Classes are shown in Table III which gives the amount of carcase, fat, pluck and skin of the various breeds on slaughter. These results have been treated so as to eliminate the differences due to age and Table IV shows the comparative weights at 9 and 21 months old.

All the records available have been given but it will be seen that in many breeds so few individuals have been exhibited that the results cannot be relied on with certainty.

Comparing the Live weights given in the third column of this table with those given in Table II it will be seen that sheep entered for the Carcase Competition are very much smaller than those entered for the Live Classes. The reason for this is possibly that the "Live" classes excite the most interest and offer the largest prize and consequently attract the best specimens of the breed. A more probable explanation however is that the "Live" exhibits are too fat to show a good carcase and so are not entered in the Carcase Competition; Long<sup>(8)</sup> has criticised the Show for this reason. As the Show is essentially a butchers' show and the animals are not designed for breeding afterwards it would appear to be a great mistake if the "Live" classes are not judged from a butchers point of view.

Inspection of Table IV will show that impressions of relative size of the breeds obtained from the live weights have to be modified when the Carcase Classes are compared. This is due to the fact that the proportion of carcase to live weight varies considerably with the breed.

Table III. *The average carcase weights of sheep shown at Smithfield, 1896-1913.*

[illegible]

Table IV. *Estimated comparative carcass weights of different breeds of sheep.*

Weights in lbs. and decimals of a lb. Calculated from Table III by correcting for differences of age.

	9 MONTHS OLD							21 MONTHS OLD						
	No. of sheep	Live weight	Carcass weight	Fat	Pluck	Skin	"Unac- counted for"	No. of sheep	Live weight	Carcass weight	Fat	Pluck	Skin	"Unac- counted for"
BRED														
Leicesters	1	144.0	89.4	5.8	4.6	17.4	26.8	2	199.6	130.5	9.1	5.6	19.1	35.3
Border Leicesters	4	130.3	77.3	4.8	4.0	14.8	29.4	—	—	—	—	—	—	—
Lincoln	9	160.5	93.3	6.6	5.4	23.9	31.4	2	290.3	193.7	14.8	7.1	29.2	45.5
Cotswold	1	179.0	120.0	6.0	6.0	17.0	36.0	—	—	—	—	—	—	—
Devon	2	153.2	94.4	9.0	3.1	18.0	26.7	3	255.1	174.7	10.7	6.1	29.0	34.6
Wensleydale	5	136.8	76.4	3.2	3.5	16.6	29.1	3	186.5	117.0	7.7	5.9	17.9	37.8
Kent	11	149.5	90.2	8.3	5.2	20.1	25.7	3	221.5	139.6	9.3	6.0	24.9	37.8
Cheviot	67	126.9	75.8	6.9	4.1	14.5	25.6	76	151.7	97.3	9.5	4.4	14.7	25.8
Blackface	16	135.9	86.5	9.4	3.9	17.8	18.3	33	170.0	108.8	14.2	4.1	18.5	24.1
Wash Mountain	2	94.8	60.4	5.7	3.2	9.6	15.9	20	123.4	78.1	9.6	3.3	11.1	23.1
Hackwick	—	—	—	—	—	—	—	1	152.3	98.3	10.8	4.7	12.6	20.9
Exmoor Horn	1	95.0	51.0	3.5	3.5	11.2	25.8	2	146.5	92.0	7.8	5.0	16.3	25.4
Dartmoor	1	133.7	80.2	7.2	4.6	14.4	27.3	2	177.3	113.7	10.2	5.1	16.8	31.5
Kerry Hill	2	124.9	75.7	5.3	4.4	15.0	24.5	5	246.0	165.9	13.5	6.7	23.6	36.3
Shropshire	3	189.4	114.8	10.2	5.2	22.4	46.8	1	246.0	170.0	10.0	6.5	25.0	32.5
Oxford Down	3	189.4	114.8	10.2	5.2	22.4	46.8	1	246.0	170.0	10.0	6.5	25.0	32.5
Hampshire	84	140.4	86.0	8.0	4.7	13.4	28.3	35	198.4	131.6	12.5	5.9	15.5	32.5
Suffolk	154	149.7	92.4	9.2	4.9	13.7	29.5	61	187.0	121.2	12.5	5.7	14.2	34.3
Southdown	80	112.7	70.4	6.2	3.8	10.5	21.8	57	137.6	90.8	7.8	4.0	11.9	23.1
Ryeland	2	131.4	80.7	7.6	4.3	14.1	24.7	2	177.2	112.5	11.4	6.1	15.8	31.4
Dorset Horn	10	133.3	84.4	7.3	4.1	12.1	25.4	2	203.8	138.6	16.6	6.2	15.0	27.4
Norfolk Horned	1	114.0	71.0	9.0	3.5	9.5	21.0	5	159.7	104.2	12.2	4.6	11.5	27.2
Murat	1	—	—	—	—	—	—	1	89.5	54.3	9.9	3.5	7.0	14.8

## Growth and Development of Sheep

Table V. *Proportions of different organs and tissues in sheep.*  
 Given in percentage of live weight. Calculated from Table IV.

BREED	No. of sheep	9 MONTHS OLD						21 MONTHS OLD					
		Carcass	Fat	Pluck	Skin	"Unac- counted for"	No. of sheep	Carcass	Fat	Pluck	Skin	"Unac- counted for"	No. of sheep
Leicesters	1	62.08	4.03	3.19	12.08	18.62	2	65.19	4.61	2.43	9.77	17.63	2
Border Leicesters	1	59.32	3.68	3.07	11.35	22.58	—	66.40	5.07	—	10.19	15.82	—
Lincoln	9	58.07	4.11	3.36	14.89	19.57	—	—	—	—	—	—	—
Cotswold	1	67.04	4.35	3.35	9.50	16.76	—	—	—	—	—	—	—
Devon	2	61.62	3.87	3.33	11.75	17.43	3	68.39	4.22	2.42	11.37	13.69	3
Wensleydale	5	58.11	3.97	2.68	12.69	22.25	5	62.70	4.14	3.13	9.67	20.26	5
Kent	11	60.33	5.62	3.48	13.44	17.13	3	63.06	5.95	2.76	11.33	16.90	3
Cheviot	97	59.73	5.44	3.24	11.43	20.16	76	63.88	6.05	2.86	9.81	17.40	76
Blackface	16	63.65	6.92	2.87	13.10	13.46	33	63.90	8.23	2.61	11.01	14.25	33
Welsh Mountain	2	63.71	6.01	3.37	10.13	16.78	20	62.35	7.28	2.79	8.91	18.67	20
Herdwick	—	—	—	—	—	—	1	64.05	7.59	3.35	9.21	15.80	1
Exmoor Horn	—	—	—	—	—	—	1	65.86	8.20	3.44	9.47	13.03	1
Dartmoor	1	53.68	3.68	3.68	11.79	30.17	2	62.91	5.30	3.46	11.14	17.19	2
Kerry Hill	1	59.59	3.39	3.44	10.77	20.41	2	64.70	5.80	2.92	9.63	17.15	2
Shropshire	2	60.61	4.24	3.52	12.01	19.62	5	65.97	5.55	2.68	9.54	16.26	5
Oxford Down	2	60.61	3.38	2.69	11.77	19.55	1	69.67	4.10	2.66	10.25	17.32	1
Hampshire	84	61.25	3.70	3.35	9.54	20.16	35	66.55	6.33	2.98	7.72	16.42	35
Suffolk	154	62.52	5.15	3.27	9.15	19.71	61	64.43	6.60	3.02	7.64	18.31	61
Southdown	80	62.55	5.80	3.37	9.32	18.25	57	65.83	5.68	2.87	8.63	16.99	57
Ryeland	2	61.42	5.78	3.27	10.73	18.80	2	63.39	6.36	3.40	9.00	17.85	2
Dorset Horn	10	63.32	5.43	3.08	9.08	19.04	2	67.43	7.83	3.00	7.55	14.19	2
Norfolk Horned	1	62.23	7.90	3.07	8.33	18.42	1	65.00	7.64	2.91	7.28	17.17	1
Murat	—	—	—	—	—	—	1	60.93	10.93	3.87	7.63	16.64	1
Average of all breeds	456	61.04	5.73	3.28	10.07	19.88	322	64.66	6.40	2.96	8.96	17.02	322

The proportions of the various organs have been calculated as a percentage of the live weight and are shown in Table V. Only those breeds which have been averaged from a sufficient number of animals to justify conclusions as to their merit, and printed in roman type in the table, are referred to below.

Taken as a whole the Southdown shows the best carcase percentage but it is closely followed by the Suffolk and Hampshire which are practically equal at 9 months although the Hampshire shows superiority at 21 months of age. The Cheviot kills at some 2 per cent. lower carcase percentage both at 9 and 21 months of age, this loss being chiefly due to the skin which is proportionately higher.

One rather unexpected finding is the low skin percentage of the Suffolk, less than that of the Southdown; this is probably accounted for by the difference in size of the breeds (see "Correlation" below).

The proportion of fat in each breed is not altogether in accordance with expectations as it is highest in the Blackface, Welsh Mountain and Suffolk and is low in the Cheviot and Southdown. It should be remembered however that this fat is "internal fat" and is not necessarily associated beyond a certain limit with the amount in the carcase; indeed there is no relation between it and the carcase percentage in the different breeds.

The carcase percentages shown in this table are much above those quoted by Diffloth<sup>(9)</sup> for French breeds.

**Age.** It is well known that as animals grow older the velocity of their growth diminishes. Ritzman<sup>(10)</sup> found that lambs in the first three months of their life made 50 per cent. of their growth during the first year, in the second three months 20 per cent., in the third three months 20 per cent. and in the fourth three months under 5 per cent. Murray<sup>(11)</sup> has expressed the velocity of growth by a formula. If the growth is calculated in lbs. per week gained however it will be found that in sheep from birth to maturity there is first an increase in the rate and then a decrease<sup>1</sup>. The period of increase however lies well within the age of 9 months at which sheep are exhibited at Smithfield; so that in comparing the rate of growth of sheep of 9 months and 21 months it will be seen that, without exception, the rate of growth diminishes considerably in the second year of life.

Columns in Table II show the rate of growth of the various breeds at 9 and 21 months in lbs. per week.

<sup>1</sup> It is hoped to publish shortly the data on which this statement is based.

The extent to which the rate falls off in the second year of life, on the average of all breeds from 4.7 lbs. per week between birth and 9 months to 1.7 lbs. per week between 9 and 21 months, would make it appear highly improbable that the gain was economic, although definite conclusions should be reserved until the ultimate composition of the gain is determined.

The effect of age on the proportions of the body in sheep is to be seen at the bottom of Table V which shows the averages for all breeds. The effect of increasing age is to increase the carcass percentage from 61 per cent. at 9 months to almost 65 per cent. at 21 months and with it the proportion of fat from 5.7 per cent. at 9 months to 6.4 per cent. at 21 months.

Table VI. *Sheep—relative development of body at different ages.*

• Weight at 9 months old expressed as a percentage of the weight at 21 months old.  
Calculated from Table IV.

BREED	9 MONTHS OLD					"Unac- counted for"
	Live weight	Carcass weight	Fat	Pluck	Skin	
Suffolk	79.6	76.2	73.6	86.0	96.4	86.0
Southdown	81.9	77.5	79.5	95.0	88.2	94.4
Hampshire	70.8	65.3	64.0	79.6	86.5	86.0
Cheviot	83.6	77.9	72.6	93.2	98.6	99.2
Average of four breeds	79.0	74.2	72.4	88.4	92.4	91.4
Suffolk ♂ × Cheviot ♀	76.2	69.4	55.3	79.2	93.2	102.1

On the other hand as the animal grows older the proportions of the pluck diminish from 3.3 to 3.0 per cent., skin from 10.1 to 9.0 per cent. and the amount "Unaccounted for" (alimentary canal etc.) from 19.9 to 17.0 per cent.

When the different ages of individual breeds are compared the same variations will be seen. The effect of age on the proportions of the body in crossbreds (see Table XIII) also confirms these conclusions.

Additional evidence is also available and is shown in Table VI in which the weight of the part at 9 months has been expressed as a percentage of the weight at 21 months.

This table shows that the skin and the alimentary canal reach their maximum development first, the pluck then attains its maximum, followed by the carcass and lastly the fat; although apparently there are variations for each breed.

This is in agreement with conclusions of Donaldson<sup>(12)</sup> who found that organs in the rat could be grouped according to the manner of their

growth, some with early rapid growth, others with a nearly uniform growth and still others which had a rapid growth just preceding puberty. Jackson and Lowrey<sup>(13)</sup> in the rat have shown that the maximum rate of growth occurs in different parts of the body at different times; the wave of most active growth passing from head to tail with advancing age.

Kern and Wattenberg<sup>(14)</sup> investigated the effect of age on the growth of sheep between the ages of 6 and 28 months.

Auernheimer<sup>(15)</sup> compared the organs of lambs of various ages with old sheep and found that the relation of rumen to abomasum at 3 weeks old was 1:2 whereas at 3 months old it was 3.5:1 and at 5 months 5:1.

The conclusions expressed above are also in accordance with Jackson and Lowrey's<sup>(13)</sup> investigations on the rat as they found that the proportions of carcass (combined skeleton and muscles) increased with age, and that the proportions of skin and viscera, although at first rising slightly, eventually decreased with age. Their results also bring out the further point, which it is important to investigate in domestic animals, namely, that with increasing age the proportion of skeleton falls while that of the muscles increases<sup>1</sup>.

**Early Maturity.** The exact definition of early maturity is difficult to define; it would appear to be as Punnett and Bailey<sup>(16)</sup> have suggested, a complex character depending on two or three factors. The *first factor* is undoubtedly weight for age. It will not depend on the absolute weight at an early age as obviously a Welsh Mountain sheep however long it lives will never attain the weight of a Lincoln at 9 months old. Maturity will depend however on the rate at which animals attain full weight. The mature weight of sheep has been taken to be represented by the weight at 21 months and the relative development of the various breeds at 9 months has been shown as a percentage of it (see Table VII). Thus ratios in this table are values for the first factor in early maturity. The various breeds stand in order of merit as regards this factor.

The ratio may be unduly influenced by two causes: (*a*) the propensity some breeds have for putting on a lot of fat late in life may cause the ratio to be unduly small when fat animals are compared, and (*b*) the effect of a "store" period decreasing the weight at 21 months; this might occur in mountain breeds where sheep designed for the 9 months class would be forced in the lowlands and those designed for the 21

<sup>1</sup> A certain amount of evidence as regards sheep has already been collected by the author and the results so far confirm the conclusions of these investigations.



months class would be left on the hills at a time when rapid growth was taking place; thus causing reduced weight at 21 months (see "Season" below).

Table VII. *Sheep—relative weights at different ages.*

The weight at 9 months old is shown as a percentage of that at 21 months old.

BREED	9 MONTHS OLD		21 MONTHS OLD	
	No. of sheep	Weight	No. of sheep	Weight
Cheviot	174	74.5	306	100
Blackface	165	70.6	309	100
South Devon	48	69.7	21	100
Devon Longwool	156	69.6	96	100
Southdown	963	68.3	660	100
Ryeland	3	67.3	117	100
Suffolk	366	66.8	219	100
Shropshire	273	64.7	339	100
Hampshire	744	64.6	429	100
Border Leicester	27	63.9	30	100
Cotswold	99	63.5	57	100
Oxford Down	303	63.3	255	100
Kent	357	62.8	361	100
Leicester	246	61.6	267	100
Dorset Horn	183	60.8	78	100
Lincoln	201	60.6	222	100

Speaking generally it will be noticed in Table VII that, with the striking exception of the Devon breeds, the Longwools come at the bottom of the table; the Down breeds led by the Southdown being more early maturing. The high position of Cheviot and Blackface sheep may possibly be affected by influence (b).

The *second factor* in early maturity is probably the rate at which the "carcase" develops. As has already been shown the proportions of the carcase to live weight are increased as the animal grows older and early maturity means that the rate of development of the carcase is hastened.

The weights of the components of a sheep at 21 months old have been taken to represent the mature condition and the relative development of the various parts at 9 months has been shown as a percentage of it (Table VI). Whereas the live weight of a Suffolk at 9 months is 79 per cent. of its weight at 21 months, the carcase weight is only 76 per cent., a difference of 3 per cent. In the Hampshire however the live weight is 71 per cent. and the carcase weight 65 per cent., a difference of 6 per cent.; thus not showing such a good factor for early maturity.

The *third factor* in early maturity is probably the rate of muscle and fat development when compared with bone in the carcase, but this as stated above has not been investigated on account of the expense and labour involved.

This factor would correspond with Armsby's(17) view that a mature animal is one in which the increase of protein tissue has reached its natural limit and that animals which reach their natural limits first are early maturing.

The factors for early maturity formulated above are more or less in agreement with the views of Müller(18) who states that early maturity has no relation to the weight or volume of the digestive organs; that early maturity resulting from better feeding in a favourable environment leads to increase in size of the body and shortening of the limbs. In our view the appearance of "shortening of the limbs" is due rather to greater muscular development giving that appearance.

As to the causes of early maturity he states that it is not exclusively dependent on nutrition but on the activity of the internal secreting glands. In this connection the changes in rate of growth of the thymus and reproduction glands relative to body weight as determined by Jackson(19) and Hatai(20) are very interesting; the proportion of the former first increasing and then decreasing with advancing age, the commencement of the decrease coinciding with the sexual maturity of the animal. Malsburg(21) states that early maturing animals have cells of larger size than those which mature late.

**Crossbreeding.** Crossbreeding is extensively used in mutton production, and many experiments, such as those initiated by Winter(22) with Welsh ewes, have been made to determine the best cross to use. Table VIII shows the weights attained by the various crosses of sheep as compiled from the records of the Show. All second and doubtful crosses have been excluded from these results. Owing to differences in time of lambing the figures as they stand are not comparable, and to correct for this Table IX has been prepared. This table also shows, for purposes of comparison, the means between the parent breeds.

Many of the figures (in italic type) given in this table are not reliable on account of the small numbers of individuals from which they have been calculated and conclusions drawn from them should be tentative only.

At each age there are six crosses with a reliable average and four of these at each age are heavier than the heaviest parent; at 9 months the crosses Oxford  $\times$  Hampshire, Leicester  $\times$  Blackface, Leicester  $\times$  Cheviot and Border Leicester  $\times$  Cheviot; at 21 months the crosses Hampshire  $\times$  Oxford, Oxford  $\times$  Hampshire, Border Leicester  $\times$  Blackface, and Border Leicester  $\times$  Cheviot. All the remainder—two at each age—are heavier than the mean of the parents; at 9 months Southdown  $\times$  Suffolk and

Southdown × Hampshire; at 21 months Leicester × Blackface and Leicester × Cheviot.

Table VIII. *Average weights and ages of crossbred sheep shown at Smithfield, 1893-1913.*

Ages in months and weeks. Weight in lbs.

CROSS		UNDER 12 MONTHS OLD			OVER 12 MONTHS OLD		
		No. of sheep	Age	Weight	No. of sheep	Age	Weight
♂	♀						
Hampshire × Oxford Down		15	10-1	203	27	20-0	270
Oxford Down × Hampshire		150	10-0	212	123	21-3	294
Hampshire × Southdown		18	9-3	178	—	—	—
Southdown × Hampshire		123	10-0	187	9	21-1	260
Hampshire × Suffolk		6	10-1	194	—	—	—
Suffolk × Hampshire		6	—	198	—	—	—
Hampshire × Shropshire		3	10-0	200	—	—	—
Shropshire × Hampshire		6	10-0	183	—	—	—
Suffolk × Border Leicester		3	8-2	215	3	20-1	338
Border Leicester × Suffolk		3	8-3	157	—	—	—
Southdown × Dorset Down		6	10-0	190	6	22-0	265
Dorset Down × Southdown		6	9-3	191	—	—	—
Leicester × Blackface		24	7-3	150	45	19-3	237
Leicester × Cheviot		45	8-0	161	36	19-3	263
Border Leicester × Blackface		—	—	—	21	19-3	245
Border Leicester × Cheviot		69	8-0	159	102	19-3	261
Cotswold × Suffolk		3	9-2	201	—	—	—
Devon Longwool × Dorset Horn		9	10-3	217	—	—	—
Cheviot × Blackface		—	—	—	3	19-0	193
Wensleydale × Blackface		9	8-0	143	—	—	—
Southdown × Kent		15	8-2	159	6	20-2	227
Southdown × Welsh		3	9-1	112	—	—	—
Southdown × Suffolk		42	9-3	186	—	—	—
Southdown × Cheviot		12	8-0	123	—	—	—
Hampshire × Lincoln		6	10-0	203	3	—	278
Hampshire × Kent		15	9-2	170	3	20-1	251
Hampshire × Devon Longwool		12	9-1	204	—	—	—
Hampshire × Cheviot		3	7-3	132	—	—	—
Suffolk × Leicester		3	8-3	219	—	—	—
Suffolk × Lincoln		—	—	—	15	21-0	266
Suffolk × Cheviot		12	8-0	140	3	19-0	214
Shropshire × Border Leicester		6	9-2	206	—	—	—
Shropshire × Kent		6	8-3	136	—	—	—
Shropshire × Cheviot		3	7-3	116	—	—	—
Shropshire × Roscommon		—	—	—	3	21-0	243
Shropshire × Dorset Horn		6	10-0	208	—	—	—
Oxford Down × Suffolk		3	10-2	218	6	21-1	306

The reason for the increased weight of crossbreds is in doubt. It may be that with pure bred sheep the best would be kept as rams whereas with crossbreds the best would come to the Show. If this is so one would expect to find the same thing happening with cattle, but it has been shown that none of the cattle crosses exceed the heavier of the parent breeds(23). Another explanation is that sheep tend to be more susceptible to the harmful effects of inbreeding than cattle. Such

harmful effects have been shown to occur in maize by East and Hayes(24); the effect of crossing in which is increased weight and vigour caused by bringing together factors controlling size which had been bred out by close breeding. Heape(25) has shown that some breeds of sheep are particularly susceptible to inbreeding.

Table IX. *Estimated comparative weights and rates of growth of crossbred sheep.*  
Weights in lbs. and decimals of a lb. Calculated from Table VIII by correcting for age differences.

CROSS	9 MONTHS OLD			21 MONTHS OLD			MEAN WEIGHT BETWEEN PURE BREEDS FOR COMPARISON	
	No. of sheep	Weight	Weekly rate of growth between birth and 9 months	No. of sheep	Weight	Weekly rate of growth between 9 and 21 months	9 months old	21 months old
♂ Hampshire × Oxford Down	15	178.3	4.95	27	276.9	1.72	175.7	274.4
♀ Oxford Down × Hampshire	150	190.8	5.30	123	288.8	1.74	—	—
Hampshire × Southdown	18	164.2	4.56	—	—	—	156.9	237.2
Southdown × Hampshire	123	168.2	4.67	9	258.4	1.62	—	—
Hampshire × Suffolk	6	170.5	4.73	—	—	—	182.0	—
Suffolk × Hampshire	6	173.0	4.83	—	—	—	—	—
Hampshire × Shropshire	3	180.0	5.00	—	—	—	167.2	—
Shropshire × Hampshire	6	164.6	4.57	—	—	—	—	—
Suffolk × Border Leicester	3	227.6	6.32	3	345.9	2.64	172.7	263.7
Border Leicester × Suffolk	3	161.5	4.49	—	—	—	—	—
Southdown × Dorset Down	6	171.0	4.75	6	255.8	1.56	146.9	—
Dorset Down × Southdown	6	176.3	4.90	—	—	—	—	—
Leicester × Blackface	24	174.0	4.84	45	246.0	1.81	160.4	245.1
Leicester × Cheviot	45	181.1	5.03	36	273.8	2.17	166.9	248.4
Border Leicester × Blackface	—	—	—	21	255.6	2.13	—	228.5
Border Leicester × Cheviot	69	179.0	4.97	102	271.8	2.17	159.6	231.8
Cotswold × Suffolk	3	199.4	5.29	—	—	—	187.8	—
Devon Longwool × Dorset Horn	9	182.0	5.05	—	—	—	168.7	—
Cheviot × Blackface	—	—	—	3	205.9	1.50	—	213.5
Wensleydale × Blackface	9	161.0	4.47	—	—	—	—	—
Southdown × Kent	15	168.4	4.68	6	229.8	1.42	153.7	235.9
Southdown × Welsh	3	109.0	3.03	—	—	—	—	—
Southdown × Suffolk	42	171.7	4.77	—	—	—	162.5	—
Southdown × Cheviot	12	138.2	3.84	—	—	—	149.4	—
Hampshire × Lincoln	6	182.7	5.07	3	272.2	1.56	187.2	299.9
Hampshire × Kent	15	161.0	4.47	3	256.6	1.88	173.3	272.0
Hampshire × Devon Longwool	12	198.5	5.51	—	—	—	177.0	—
Hampshire × Cheviot	3	153.2	4.26	—	—	—	169.0	—
Suffolk × Leicester	3	225.3	6.26	—	—	—	180.0	—
Suffolk × Lincoln	—	—	—	15	266.0	1.69	—	303.5
Suffolk × Cheviot	12	157.6	4.37	3	225.1	1.68	174.5	248.7
Shropshire × Border Leicester	6	195.2	5.42	—	—	—	157.9	—
Shropshire × Kent	6	139.9	3.89	—	—	—	164.0	—
Shropshire × Cheviot	3	134.5	3.74	—	—	—	159.7	—
Shropshire × Roscommon	—	—	—	3	243.0	1.84	—	—
Shropshire × Dorset Horn	6	187.2	5.20	—	—	—	158.2	—
Oxford Down × Suffolk	3	187.0	5.19	6	304.0	2.05	181.0	278.0

Both Humphrey and Kleinheinz(26) and Mumford(27) have found that it is the size of the ewe and not the size of the ram which determines the size of the lamb. Inspection of the weights attained by the reciprocal cross—Hampshire-Southdown—however show that there is very little difference in weight at 9 months of age.

The reciprocal cross—Hampshire  $\times$  Oxford—is interesting; the cross Oxford ram and Hampshire ewe having both the early maturity of the Hampshire at 9 months and the larger weight of the Oxford at 21 months, whereas the cross Hampshire ram and Oxford ewe has neither of these qualities. It may be that as Punnett and Bailey<sup>(28)</sup> have shown in rabbits, size and early maturity are inherited independently and may be sex-linked; or it may be that the Hampshire ewe is a particularly good mother for it has been shown by Ritzman<sup>(29)</sup> that the growth of the lamb is influenced to a very large extent by the milk supply of the ewe. All the other Hampshire crosses (with the exception of that with the Shropshire) show the same thing, the Hampshire ewes giving larger lambs than the reciprocal cross.

Table X. *Crossbred sheep—relative weights at different ages.*

The weight at 9 months old is shown as a percentage of that at 21 months old.

CROSS		9 MONTHS OLD		21 MONTHS OLD	
♂	♀	No. of sheep	Weight	No. of sheep	Weight
Hampshire $\times$ Oxford Down		15	64.39	27	100
Oxford Down $\times$ Hampshire		150	66.07	123	100
Southdown $\times$ Hampshire		123	65.09	9	100
Oxford Down $\times$ Suffolk		3	61.51	6	100
Southdown $\times$ Dorset Down		6	71.61	6	100
Southdown $\times$ Kent		15	73.28	6	100
Hampshire $\times$ Kent		15	62.74	3	100
Hampshire $\times$ Lincoln		6	67.12	3	100
Suffolk $\times$ Cheviot		12	69.92	3	100
Leicester $\times$ Blackface		24	70.73	45	100
Leicester $\times$ Cheviot		45	66.14	36	100
Border Leicester $\times$ Cheviot		69	65.85	102	100

If Table X is compared with Table VII it will be seen that in two certain (Oxford  $\times$  Hampshire, Leicester  $\times$  Blackface) and in three doubtful cases (Southdown  $\times$  Dorset Down, Southdown  $\times$  Kent, Hampshire  $\times$  Lincoln) the rate of early maturity (factor one) is increased above the level of the highest parent breed, which seems to indicate that crossing tends to increase early maturity.

The relative development of the body in the various crosses of sheep is shown in Table XI which has been compiled direct from the records of the Show. Age differences have been eliminated and the comparative averages are shown in Table XII. When this table is compared with Table IX it will be noticed that the live weights of the crosses are much less in the "Carcase" than they are in the "Live" classes.

The proportions of the various organs are given as percentages of the live weight in Table XIII. Unfortunately only a few of the crosses

Table XI. The average carcass weights of crossbred sheep shown at Swinfield, 1896-1913.  
Weights in lbs. and decimals of a lb. Ages in months and weeks.

OVER 12 MONTHS OLD															
Cross	No. of sheep	Age	Live weight	Carcass weight	Fat	Skin	"Unac- counted for"	No. of sheep	Age	Live weight	Carcass weight	Fat	Pluck	Skin	"Unac- counted for"
♂															
♀															
Suffolk x Shropshire	2	7-3	150.0	93.5	8.2	4.0	15.0	20-3	—	—	—	—	—	—	—
Suffolk x Norfolk Horn	1	8-2	144.0	88.0	8.5	4.0	13.0	30.5	—	—	—	—	—	—	—
Suffolk x Kent	1	7-2	103.0	62.0	4.0	4.0	12.5	20.5	—	—	—	—	—	—	—
Suffolk x Wensleydale	1	7-3	135.0	76.0	7.5	4.0	11.5	36.0	—	—	—	—	—	—	—
Suffolk x Leicester	1	8-0	177.0	118.0	8.0	5.0	18.0	28.0	—	—	—	—	—	—	—
Suffolk x Blackface	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Suffolk x Southdown	1	8-3	130.0	85.0	9.0	4.0	11.0	21.0	19-2	166.5	106.5	16.2	4.2	14.2	25.4
Southdown x Suffolk	17	9-2	136.4	86.5	8.2	4.0	13.1	14.6	20-0	141.0	86.0	7.0	4.2	11.7	32.1
Suffolk x Oxford Down	1	8-0	102.0	61.0	6.0	3.0	13.0	19.0	—	—	—	—	—	—	—
Oxford Down x Suffolk	4	8-1	127.0	78.7	6.9	4.4	13.1	23.9	20-1	146.0	83.0	7.5	5.0	12.5	33.0
Suffolk x Hampshire	1	8-2	116.0	69.0	5.2	4.2	10.7	26.4	1	—	—	—	—	—	—
Hampshire x Suffolk	1	9-0	133.0	82.0	7.5	4.5	14.5	24.5	—	—	—	—	—	—	—
Suffolk x Lincoln	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lincoln x Suffolk	1	7-2	136.0	87.0	8.0	4.5	15.0	21.5	1	21-0	258.0	170.0	16.7	9.5	22.0
Suffolk x Cheviot	31	8-0	116.0	69.2	6.1	3.7	12.3	24.7	26	20-0	167.0	107.4	11.8	5.2	14.6
Cheviot x Suffolk	1	8-0	135.0	78.0	7.5	4.0	10.5	35.0	2	20-0	156.0	101.0	17.5	4.2	11.2
Southdown x Shropshire	—	—	—	—	—	—	—	—	3	20-0	136.3	75.0	8.3	4.1	9.3
Southdown x Kent	3	9-0	125.7	69.3	5.0	4.0	20.3	27.1	3	19-3	158.7	98.7	9.7	5.2	17.3
Southdown x Welsh	4	8-0	86.5	52.5	6.1	3.0	9.2	15.7	6	20-1	114.0	71.0	8.3	3.9	11.7
Southdown x Cheviot	7	7-3	103.0	63.1	5.9	3.6	11.7	18.7	8	19-3	138.4	87.2	8.9	4.2	13.4
Southdown x Blackface	1	7-0	82.0	47.0	4.0	3.0	9.0	19.0	1	19-2	100.0	60.0	6.0	6.0	16.0
Southdown x Border Leicester	1	8-1	131.0	79.0	7.0	4.5	14.5	26.6	1	20-1	176.6	115.0	12.0	5.5	14.0
Southdown x Norfolk Horn	4	9-0	117.0	71.0	6.2	4.0	13.2	22.6	1	20-3	159.5	99.7	10.4	4.5	13.6
Norfolk Horn x Southdown	—	—	—	—	—	—	—	—	1	21-0	161.0	109.0	10.0	5.0	12.0
Southdown x Hampshire	7	9-3	148.4	97.6	8.3	4.7	14.3	23.5	—	—	—	—	—	—	—
Hampshire x Southdown	4	9-0	119.7	76.7	7.5	4.1	13.7	17.7	1	23-3	176.0	120.0	10.0	4.5	15.5
Hampshire x Dorset Horn	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hampshire x Border Leicester	1	8-1	99.0	59.0	3.0	3.0	15.0	19.0	—	—	—	—	—	—	—
Hampshire x Cheviot	1	8-2	113.0	88.0	8.5	4.5	16.5	25.5	—	—	—	—	—	—	—
Shropshire x Hampshire	—	—	—	—	—	—	—	—	1	20-2	165.0	66.0	7.0	4.0	9.0
Shropshire x Welsh	—	—	—	—	—	—	—	—	1	19-3	254.0	172.0	12.0	7.0	19.0
Shropshire x Leicester	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Shropshire x Border Leicester	1	8-3	145.0	83.0	6.0	4.0	15.0	40.0	—	—	—	—	—	—	—
Oxford Down x Hampshire	2	9-3	202.5	126.5	11.0	5.5	24.5	36.0	—	—	—	—	—	—	—
Oxford Down x Cheviot	1	—	180.0	105.0	5.5	3.0	14.5	30.0	—	—	—	—	—	—	—
Leicester x Blackface	3	7-3	125.3	77.0	7.8	3.8	17.2	19.5	11	20-2	188.0	124.0	9.2	5.4	19.8
Leicester x Dorset Horn	1	10-1	136.0	85.0	7.0	4.0	16.0	24.0	—	—	—	—	—	—	—
Border Leicester x Blackface	3	7-2	121.7	74.7	7.5	4.0	13.7	21.8	3	21-0	139.7	77.7	6.1	4.2	16.0
Border Leicester x Cheviot	5	8-0	107.4	66.0	7.5	3.8	12.1	15.0	3	19-3	186.3	124.7	11.2	5.0	16.8
Border Leicester x Suffolk	3	7-3	118.7	70.7	5.3	4.7	11.8	25.2	—	—	—	—	—	—	—
Cotswold x Dorset Horn	2	11-1	160.0	95.5	9.5	6.5	16.0	32.5	—	—	—	—	—	—	—
Devon x Dorset Horn	—	—	—	—	—	—	—	—	1	23-1	243.0	166.0	24.0	5.0	18.0
Ryeland x Hampshire	1	8-0	118.0	73.0	5.0	4.0	16.0	20.0	—	—	—	—	—	—	—
Kerry Hill x Welsh	1	7-3	92.0	58.0	4.0	3.5	14.0	12.5	1	19-2	120.0	66.0	6.5	4.0	9.0
Wensleydale x Blackface	1	—	—	—	—	—	—	—	12	19-2	148.1	92.9	8.7	4.7	15.4
Hertwick x Cheviot	1	—	—	—	—	—	—	—	1	19-0	84.0	50.0	4.5	3.5	10.2



Given as percentage of live weight. Calculated from Table XII.

9 MONTHS OLD										21 MONTHS OLD			
No. of sheep	Cross	♂	Carcass weight	Fat	Pluck	Skin	"Unac- counted for"	No. of sheep	Carcass weight	Fat	Pluck	Skin	"Unac- counted for"
2	Suffolk × Shropshire	2	62.29	5.45	2.88	9.99	19.39	2	63.95	9.71	2.51	8.48	15.35
1	Suffolk × Norfolk Horn	1	61.09	5.90	2.76	8.99	21.26	2	61.03	4.94	2.98	8.32	22.73
1	Suffolk × Kent	1	60.16	3.81	3.81	12.14	20.08	2	62.09	5.59	3.26	8.38	20.68
1	Suffolk × Wensleydale	1	56.16	5.48	2.83	8.48	26.96	1	63.41	5.09	3.37	8.52	19.61
1	Suffolk × Leicester	1	66.68	4.52	2.82	10.15	15.83	1	56.61	4.72	3.54	9.45	25.68
1	Suffolk × Blackface	1	65.37	6.95	3.06	8.45	16.17	2	63.95	9.71	2.51	8.48	15.35
17	Southdown × Suffolk	17	63.42	6.03	2.94	9.50	18.02	2	61.03	4.94	2.98	8.32	22.73
1	Suffolk × Oxford Down	1	59.81	5.75	2.88	12.73	18.83	2	62.09	5.59	3.26	8.38	20.68
4	Oxford Down × Suffolk	4	61.92	5.42	3.39	10.36	19.01	1	63.41	5.09	3.37	8.52	19.61
1	Suffolk × Hampshire	1	59.44	4.88	3.58	9.20	22.90	1	56.61	4.72	3.54	9.45	25.68
1	Hampshire × Suffolk	1	61.65	5.64	3.38	10.90	18.23	1	65.89	6.47	3.68	8.52	15.44
1	Suffolk × Lincoln	1	64.05	5.83	3.31	11.04	15.77	1	65.89	6.47	3.68	8.52	15.44
31	Lincoln × Suffolk	31	58.89	5.21	3.22	10.58	22.10	26	64.29	7.02	3.08	8.73	16.88
1	Suffolk × Cheviot	1	57.65	5.53	2.90	7.77	26.15	2	64.71	7.33	2.68	7.14	18.14
1	Cheviot × Suffolk	1	57.65	5.53	2.90	7.77	26.15	2	64.71	7.33	2.68	7.14	18.14
3	Southdown × Shropshire	3	55.73	3.97	3.18	16.15	21.57	3	59.35	6.56	3.23	7.46	23.40
3	Southdown × Kent	3	60.53	6.99	3.39	10.48	18.61	3	62.18	6.11	3.26	10.91	17.54
7	Southdown × Welsh	7	61.17	5.69	3.51	11.30	18.33	8	63.02	6.39	2.99	9.65	17.65
1	Southdown × Cheviot	1	57.41	4.84	3.61	10.93	23.21	1	60.04	5.94	5.94	15.98	12.10
1	Southdown × Blackface	1	60.29	5.39	3.36	11.09	19.87	1	65.31	6.79	3.12	7.95	16.83
1	Southdown × Border Leicester	1	60.68	5.30	3.42	11.28	19.32	4	65.32	6.51	2.85	8.55	19.37
1	Southdown × Norfolk Horn	1	65.76	5.55	3.14	9.71	15.84	1	67.70	6.21	3.11	7.45	15.53
7	North Horn × Southdown	7	65.76	5.55	3.14	9.71	15.84	1	67.70	6.21	3.11	7.45	15.53
4	Southdown × Hampshire	4	64.08	6.26	3.42	9.44	16.80	1	68.21	5.71	2.57	8.86	14.65
1	Hampshire × Southdown	1	55.28	4.47	2.86	12.00	24.79	1	68.21	5.71	2.57	8.86	14.65
1	Hampshire × Dorset Horn	1	59.54	3.06	3.06	15.00	29.25	1	68.21	5.71	2.57	8.86	14.65
1	Hampshire × Cheviot	1	61.56	3.94	3.10	11.49	17.91	1	68.21	5.71	2.57	8.86	14.65
1	Shropshire × Hampshire	1	61.56	3.94	3.10	11.49	17.91	1	68.21	5.71	2.57	8.86	14.65
1	Shropshire × Welsh	1	61.56	3.94	3.10	11.49	17.91	1	68.21	5.71	2.57	8.86	14.65
1	Shropshire × Leicester	1	56.04	4.07	2.69	10.12	27.08	1	68.21	5.71	2.57	8.86	14.65
1	Shropshire × Border Leicester	1	62.14	5.43	2.72	12.04	17.67	1	68.21	5.71	2.57	8.86	14.65
2	Oxford Down × Hampshire	2	55.09	4.06	2.54	12.29	25.42	1	68.21	5.71	2.57	8.86	14.65
1	Oxford Down × Cheviot	1	61.37	6.05	3.02	13.69	15.87	11	65.94	4.88	2.85	10.49	15.84
3	Leicester × Blackface	3	61.37	6.05	3.02	13.69	15.87	11	65.94	4.88	2.85	10.49	15.84
1	Leicester × Dorset Horn	1	62.51	5.19	2.93	11.80	17.37	3	55.62	4.37	3.01	11.45	25.55
1	Border Leicester × Blackface	1	61.40	6.16	3.26	11.26	17.92	3	55.62	4.37	3.01	11.45	25.55
1	Border Leicester × Cheviot	1	59.22	4.46	3.47	11.34	22.44	3	55.62	4.37	3.01	11.45	25.55
2	Border Leicester × Suffolk	2	59.22	4.46	3.47	11.34	22.44	3	55.62	4.37	3.01	11.45	25.55
2	Border Leicester × Hampshire	2	59.22	4.46	3.47	11.34	22.44	3	55.62	4.37	3.01	11.45	25.55
2	Cotswold × Dorset Horn	2	59.70	6.00	4.13	10.06	20.11	1	68.49	5.93	2.69	7.46	12.03
1	Devon × Dorset Horn	1	61.82	4.22	3.39	13.55	17.02	1	68.49	5.93	2.69	7.46	12.03
1	Ryeland × Hampshire	1	61.82	4.22	3.39	13.55	17.02	1	68.49	5.93	2.69	7.46	12.03
1	Kerry Hill × Welsh	1	62.92	4.31	3.74	15.17	13.56	1	57.41	5.43	3.33	7.52	26.28
1	Wensleydale × Blackface	1	62.92	4.31	3.74	15.17	13.56	12	62.67	5.80	3.14	10.39	18.00
1	Herdwick × Cheviot	1	62.92	4.31	3.74	15.17	13.56	1	59.48	5.28	4.09	12.07	19.08
121	Average of all Crosses	121	60.70	5.28	3.23	10.76	20.03	100	63.26	5.60	2.77	9.44	18.93



have been exhibited in sufficient numbers for reliable averages to be given; the remainder shown in italic type are of use only as an indication of what the true result would be, and are not referred to below.

The averages for all crossbred sheep are shown at the bottom of this table and if they are compared with the averages of pure bred sheep shown at the bottom of Table V it will be seen that the proportion of carcase in crossbred sheep is not so high as that of the pure breeds; this applies to sheep of both 9 months and 21 months old. The proportion of fat and pluck is also less but the proportion of skin and alimentary canal ("Unaccounted for") is greater in crossbred animals at both ages.

When one comes to compare individual crosses in this way however it is found that at 9 months old the Southdown-Suffolk cross has a higher carcase percentage, but the Suffolk-Cheviot cross has less than either of the pure breeds. At 21 months old the Leicester-Blackface cross has a higher carcase percentage than either pure breed and the Suffolk-Cheviot cross has one slightly higher than the average of the two breeds.

Unfortunately only a few figures are available for a comparison of crosses as regards early maturity (factor 2); the cross Suffolk-Cheviot being the only one for which figures exist. Table VIII shows that in this case the cross is not so early maturing as either of the parent breeds.

**Variation.** One of the qualifications a pure breed should possess is that it should breed true to type—as regards size, that it should maintain

Table XIV. *Variation in live weight of sheep shown at Smithfield, 1893–1913.*

The sheep have been shown in pens of three so that the figures shown in the table are deviations and coefficients on the average of pens of three.

BREED	STANDARD DEVIATION				COEFFICIENT OF VARIABILITY		
	9 months old		21 months old		9 months old	21 months old	Breed average
	No. of sheep	lbs.	No. of sheep	lbs.			
Leicester	246	14.60	267	19.80	8.47	7.07	7.77
Southdown	963	11.43	660	14.77	8.32	7.34	7.83
Hampshire	744	13.05	429	19.16	7.37	7.01	7.19
Suffolk	366	17.70	219	16.40	9.43	5.84	7.63
Average of four breeds	4	14.19	4	17.53	8.40	6.81	—
Cheviot	174	15.30	306	26.50	9.47	12.22	10.84
Blackface	165	13.80	309	21.77	9.29	10.35	9.82
Southdown ♂ × Hampshire ♀	123	13.70	—	—	8.15	—	—
Leicester ♂ × Cheviot ♀	45	10.60	36	23.20	5.83	8.47	7.15

a standard size with little variation. Owing to a variety of causes however, some of which have been investigated, there is considerable indi-

vidual variation occurring within a breed. As a measure of the variation which occurs the coefficient of variability has been calculated for a few breeds and crosses. The last column of Table XIV shows that in the Leicester, Southdown, Hampshire and Suffolk breeds there is very little difference in variability. As might be expected however the Cheviot and Blackface breeds show greater variability, owing possibly to differences in conditions under which they are kept or probably to the relative unimprovement of some of the strains of these breeds.

It is remarkable that crossbreds—the first cross—are not more variable than the parent pure breeds, even when the breeds differ considerably in size.

Variability in weight decreases with age in the Down and Longwooled breeds (see Table XIV). The average of four breeds shows a coefficient of variability at 9 months of 8.4 whereas at 21 months old it is only 6.8. This result is contrary to the findings of Hammond<sup>(30)</sup> in cattle and Robertson<sup>(31)</sup> in children, who found an increase in variability with age, but is in accordance with Jackson's<sup>(32)</sup> results in rats. The latter found that the variability of body weight increases up to 20 days old but then decreases with advancing age. King<sup>(33)</sup> found with rats over 3 months old that there was a correlation between the rapidity of growth and variability of body weight. The reason for the increased variability in these breeds of sheep at 9 months old as compared with 21 months may be due to the variation in the rate at which young animals put on fat; this conclusion is confirmed by the variations which occur in the proportions of the carcase (see below).

The Cheviot and Blackface breeds differ from the others by showing increased variability with age. The Leicester ♂-Cheviot ♀ cross also increases in variability with age. The reason why these breeds and crosses should differ from the others is probably due to the conditions under which they are kept. It seems probable that the greater variability of the Cheviot and Blackface at 21 months is due to the fact that sheep of this age have undergone differences in management in their first year of life—some kept for a "Store" period under highland conditions and others forced under lowland conditions from birth (see discussion on "Store" period under "Season" below), whereas those designed for the 9 months class would be kept growing from birth. Cattle which frequently have a "Store" period also show increased variability with age<sup>(30)</sup>.

As variation forms the basis on which the improvement of breeds is made, the effect of selection upon the variability in live weight has been

investigated. The coefficient in variability has been calculated in each of the seven-year periods and is shown in Table XV. Taking the average of four breeds—Leicester, Southdown, Hampshire, Suffolk—the results show that at 9 months old variability in weight has steadily increased from 1893 to 1913, while at 21 months old there has been very little change; this, if the above explanation of increased variability at 9 months old is accepted, being probably due to the fact that some breeders have been forcing lambs by fattening to more early maturity while others have not. This conclusion is in general agreement with the increase in weight of lambs from 1893 to 1913, shown in Table XVIII.

Table XV. *Changes in variation in live weight of sheep from 1893 to 1913.*

*Coefficient of Variability.*

Period I, 1893-1899; Period II, 1900-1906; Period III, 1907-1913.

BREED	9 MONTHS OLD						21 MONTHS OLD					
	No. of sheep	Period I	No. of sheep	Period II	No. of sheep	Period III	No. of sheep	Period I	No. of sheep	Period II	No. of sheep	Period III
Leicester	27	8.72	28	7.91	27	9.27	33	7.31	28	5.38	28	8.16
Southdown	116	7.42	106	9.33	99	9.42	82	6.55	68	8.68	70	7.04
Hampshire	106	6.19	76	8.01	66	8.41	59	7.02	54	6.75	30	7.15
Suffolk	38	9.42	51	9.96	33	8.97	26	5.97	26	6.04	21	5.60
Av. of four breeds	—	7.82	—	8.81	—	9.02	—	6.71	—	6.71	—	6.84

The variability of the proportions of the body in four breeds of sheep has been shown in Table XVI. The coefficient of variability has been used as the basis for the calculations but as objections have been raised as to its use in cases of percentages the standard deviations are also shown. From the average of the four breeds given at the bottom of the table it will be seen that the different systems of the body show great differences in the amount of variability. The proportions of fat are naturally very variable, due to the different stages of fatness at which sheep are exhibited. The amount "Unaccounted for" consisting mainly of alimentary canal and contents is also very variable, due to the period of starvation and amounts of food contained in the stomachs and intestines. The proportions of skin and pluck are rather less variable and are probably due in the former to differences in the rate of growth of the wool and in the latter to the thoroughness of bleeding, affecting heart and lung weight and the period of starvation affecting liver weight. The proportions of the carcass are the least variable in the body and are even less variable than the live weight. It is remarkable that there does

not exist more variability in the carcass due to stage of fattening, since there is such great variability in the proportions of fat. The proportions of internal fat however are not always correlated with the carcass percentage (see "Correlation") and presumably with the amount of fat in the carcass.

Table XVI. *Variation in proportional development of sheep shown at Smithfield, 1896-1913.*

BREED	9 MONTHS OLD						21 MONTHS OLD					
	No. of sheep	Carcass	Fat	Pluck	Skin	"Unac- counted for"	No. of sheep	Carcass	Fat	Pluck	Skin	"Unac- counted for"
Cheviot	67	3.36	1.38	0.54	1.58	4.38	76	3.96	1.25	0.45	1.65	4.12
Hampshire	84	3.06	1.16	0.40	1.77	3.33	35	4.74	1.47	0.51	1.21	4.48
Suffolk	154	3.34	1.37	0.49	1.60	3.78	61	3.45	1.35	0.40	1.05	3.57
Southdown	80	3.01	1.39	0.52	1.89	3.18	57	4.31	1.19	0.46	1.06	4.54
Average	4	3.19	1.32	0.48	1.71	3.67	4	4.11	1.31	0.45	1.24	4.18
(b) Coefficient of variability:												
Cheviot	67	5.62	25.37	16.66	13.82	21.72	76	6.19	20.66	15.74	16.82	23.70
Hampshire	84	5.00	20.35	11.97	18.55	16.52	35	7.12	23.22	17.01	15.67	27.31
Suffolk	154	5.41	22.27	15.04	17.48	19.07	61	5.35	20.41	13.31	13.73	19.50
Southdown	80	4.81	25.27	15.61	20.28	16.52	57	6.55	20.95	16.03	12.28	26.72
Average	4	5.21	23.31	14.82	17.52	18.46	4	6.30	21.31	15.52	14.62	24.31

The effect of age on the variability of the proportions of the body is very marked. The variability of the proportions of carcass, pluck and alimentary canal ("Unaccounted for") increases while the variability of the proportions of fat and skin (except in Cheviots) decreases with advancing age. The increase of variability with age in the carcass, pluck and alimentary canal is similar to that which occurs in cattle (30).

The reason why young sheep show greater variability of fat is probably due to the variations in early maturity brought about by feeding, and to their differences in capability to put on fat at an early age; similar changes occur in cattle.

The greater variability in the skin of young animals is probably due to the stage of development at which the wool begins to grow, some sheep developing wool at an earlier stage than others; that this is the reason is suggested by the fact that the variability of the proportions of the hide in cattle increases with age.

**Correlation.** It is well known that in the body there exists a correlation between the glands of internal secretion and that when one is removed others hypertrophy. It is known also that the development of one part is frequently correlated with the growth of another, for example the development of the corpus luteum causing growth of the mammary

gland. In the development of the sheep there is evidence that certain parts tend to be correlated in their growth and that over-development of some organs is associated with the under-development of others. As the degree of development of an organ is measured by the proportion it bears to the total weight of the animal it naturally follows that if the proportions of some go up that of others must come down. The object of the breeder will naturally be directed to increasing the proportions

Table XVII. *Correlation between different parts of the body in sheep.*

Percentage of live weight. For explanation of table see p. 373.

I. *Grouped by live weight:*

Percentage of organ or tissue grouped by	No. of sheep	9 MONTHS OLD					"Unaccounted for"	No. of sheep	21 MONTHS OLD					"Unaccounted for"
		Carcase	Fat	Pluck	Skin				Carcase	Fat	Pluck	Skin		
Highest	96	63.30	6.30	3.24	8.92	18.24		58	67.28	6.37	2.65	7.90	15.80	
High average	96	61.93	5.78	3.11	9.24	19.84		57	63.98	6.21	2.95	8.76	18.10	
Low average	96	60.73	5.46	3.37	9.95	20.49		57	62.24	6.11	3.04	8.98	19.63	
Lowest	97	60.52	5.48	3.51	10.71	19.78		57	62.28	5.86	3.36	9.15	19.35	

II. *Grouped by carcase percentage:*

Highest	96	—	6.04	3.24	9.00	16.49	58	—	6.19	2.71	8.12	14.43	
High average	96	—	6.00	3.29	9.68	17.94	57	—	6.21	3.08	8.64	16.63	
Low average	96	—	5.75	3.31	10.08	19.83	57	—	6.19	3.12	9.01	18.71	
Lowest	97	—	5.19	3.20	10.54	23.64	57	—	5.90	2.93	9.05	23.33	

III. *Grouped by fat percentage:*

Highest	96	62.60	—	3.23	9.41	17.37	58	64.19	—	2.86	8.41	16.19	
High average	96	62.21	—	3.31	9.90	18.41	57	64.49	—	3.04	8.57	17.16	
Low average	96	61.70	—	3.25	9.57	20.15	57	64.16	—	2.99	8.72	18.51	
Lowest	97	60.00	—	3.26	10.32	22.32	57	62.93	—	2.93	8.89	20.75	

IV. *Grouped by pluck percentage:*

Highest	96	61.56	5.51	—	10.23	18.59	58	63.08	6.01	—	7.18	18.47	
High average	96	62.24	5.98	—	9.70	18.58	57	63.87	6.44	—	8.48	18.21	
Low average	96	61.23	5.65	—	9.51	20.42	57	63.74	5.75	—	8.38	18.63	
Lowest	97	61.48	5.74	—	9.67	20.31	57	65.18	6.03	—	8.61	17.74	

V. *Grouped by skin percentage:*

Highest	96	60.13	5.40	3.43	—	19.00	58	62.72	5.87	3.09	—	18.14	
High average	96	61.20	5.66	3.24	—	19.86	57	63.69	6.23	2.92	—	18.33	
Low average	96	62.09	5.76	3.33	—	19.68	57	63.83	6.18	2.94	—	18.87	
Lowest	97	63.05	6.09	3.19	—	19.78	57	65.81	6.31	2.90	—	17.90	

VI. *Grouped by "Unaccounted for" percentage:*

Highest	96	57.73	5.02	3.11	9.66	—	58	59.16	5.57	2.91	8.76	—	
High average	96	61.51	5.21	3.23	9.64	—	57	63.17	5.89	3.11	8.76	—	
Low average	96	63.07	6.04	3.30	9.52	—	57	65.87	6.28	2.98	8.35	—	
Lowest	97	64.07	6.89	3.48	10.29	—	57	67.70	6.81	2.87	8.82	—	

of the carcase and reducing the amount of offal in the body and a knowledge of the correlation which exists between the carcase and other parts of the body will be of assistance to him. Correlations between the size of an animal and the proportions of its parts will also be of use in estimating the relative economy of small and large breeds and in determining

whether from an economic point of view it would be better to increase or decrease the size of a breed.

Table XVII shows the correlation of the different parts of the body by weight. It has been compiled from the results of four breeds—Southdown, Suffolk, Hampshire and Cheviot—each breed considered by itself shows much the same results as the combined ones which are printed. The methods used are explained under a description of this table above and the results have been judged to show correlation only if they run consecutively throughout the series—highest, high average, low average, lowest—and are confirmed by the reciprocal groupings.

*Live weight.* High live weight is correlated with a large proportion of carcase and fat and a small proportion of pluck, skin and alimentary canal ("Unaccounted for"); that is within a breed the heaviest animals generally have the highest carcase and fat percentage and the lowest percentage of pluck, skin, and alimentary canal; this is true both at 9 and 21 months of age.

These conclusions are in general agreement with the results of other investigators. The relation of live weight to fat, pluck and skin is similar to that found by Hammond (34) in cattle. Boycott and Damant (35) in rats discovered that the proportion of fat increases with the body weight. Seeberger (36) in sheep found that the heart and lungs are relatively larger in small than in large animals. Mancini (37) found in sheep that while the heart and liver are directly proportional to live weight the lungs are not. Mancini's conclusions are hardly borne out however when the sheep (Gafagnia breed) are grouped according to their live weight, as is shown below:

No. of sheep...	Average live weight—kilo			
	28.2	33.3	36.3	39.9
	10	9	9	10
	Percentage of live weight			
Lungs ...	1.688	1.542	1.667	1.310
Heart ...	0.653	0.685	0.616	0.645
Liver ...	2.750	2.670	2.130	2.200
Total of three organs...	5.091	4.897	4.413	4.155

From the totals of the three organs, which constitute the bulk of the pluck, it will be seen that the highest live weight is associated with the lowest proportion of pluck. Joseph (39) found that in dogs the proportions of the heart fall as the body increases in weight. In rats of different ages however Hatai (40) found that the weight of the heart is correlated with that of the body.

Joseph(41) found that small rabbits have relatively larger stomachs and caecums than larger rabbits and Livingstone(42) has confirmed these results with the whole gastro-intestinal tract and its contents. He also discovered that the proportion of alimentary canal and its contents to body weight varied at different times of the year, being greater in the autumn after the summer grass and vegetable feeding than it was in the spring after being fed through the winter on grain.

Tridon(43) and also Mancia(37) have shown that in sheep the lightest animals have the greatest proportion of bone. This is contrary to Cline's(44) conclusion that it is wrong to enlarge a breed of animals, for in proportion to the increase in size they become worse in form.

*The proportion of the carcass* is correlated with the proportion of fat but inversely with the proportion of skin and alimentary canal ("Unaccounted for") at 9 months old. The same holds true at 21 months old with the exception that the sheep with the largest carcass percentage have not the most fat.

*The proportion of fat* is correlated at 9 months old with the proportion of carcass and inversely with the proportion of skin and alimentary canal ("Unaccounted for"). At 21 months old the same holds true with the exception that the fattest sheep have not the highest carcass percentage. This exception is contrary to Lawes and Gilbert's(45) general finding that the carcass percentage of an animal rises as it increases in fatness and supports the conclusions of Mackenzie and Marshall(46) in cattle. The reason for this exception is probably that at a certain stage of fatness more fat is deposited internally than on the carcass, thus lowering the proportions of the latter. That the fattest animals have not necessarily the highest carcass weights is seen to apply when different breeds are compared, see Table V. A large proportion of fat is not necessarily associated with a large carcass percentage, for at 21 months Mountain sheep have 8.2 per cent. fat and a carcass percentage of 63.9 whereas Southdowns which contain only 5.7 per cent. of fat have a carcass percentage of 65.8.

*The proportion of pluck* (heart, lungs, liver, one thymus and some fat) does not appear to be correlated with any of the other parts of the body, except as pointed out above inversely with live weight. Owing to the non-association of pluck with the other parts of the body its weight would probably form the best estimate of the true size of the animal, as distinct from conditions of fatness, fleshing and skin development. It is known that heart and lungs shrink less than most other parts of the body in starvation although the liver shows great loss in weight(47).

The relative size (as percentage of live weight) of these organs in a four year old ram is stated by Colin<sup>(48)</sup> to be—lungs 1.6 per cent., liver 1.2 per cent. and heart 0.4 per cent. The relation of pluck to the rest of the body is complicated by variations in size of the thymus, the relation of which to body weight Jackson<sup>(49)</sup> has shown first to increase and then decrease with age. For practical purposes however the weight of this, compared with the other organs, is small.

*The proportion of skin* is correlated inversely with the proportion of carcase and fat in the body. This is similar to the correlation which exists in cattle<sup>(34)</sup>.

*The proportion of "Unaccounted for,"* consisting mainly of the digestive tract and contents, varies inversely with the proportions of carcase and fat. At 9 months old there is an indication that it is associated inversely with the proportions of pluck but this is not confirmed by the reciprocal grouping.

Speaking generally it will appear that with minor exceptions, such as the inverse relation between carcase and fat in sheep of 21 months, the proportions of carcase and fat vary together while those of skin and alimentary canal ("Unaccounted for") frequently vary together and generally in opposition to those of the former group, the proportion of pluck being quite independent of either of these two groups. These relationships are on the whole similar to those which occur in cattle<sup>(34)</sup>.

**Selection.** In order to determine the effect of selection and the "standards" set up by Breed Societies upon the size of sheep, and in order to obtain an indication of the general direction being taken in the improvement of breeds as regards size, Table XVIII has been prepared. This table gives the average weights of sheep shown in each of the three periods—I, 1893 to 1899; II, 1900 to 1906; and III, 1907 to 1913.

When the breeds are considered individually it will be seen that at 9 months old the following have increased steadily in weight—Leicester, Lincoln, Devon Longwool, Suffolk, Shropshire and Oxford Down. Kents, Cheviots and Southdowns show an increase in the second period, followed by a corresponding decrease in the third period; this increase being due possibly to the greater fatness of the animals during this period (see Table XX). The Hampshire is the only breed which shows a constant decline in weight at this age.

At 21 months old only two breeds, the Leicester and Oxford Down, show a constant increase in weight. The majority of the breeds—Lincoln, Kent, Devon Longwool, Cheviot, Hampshire, Suffolk and



Shropshire—show an increase in the second period followed by a corresponding decrease in the third period; this increase is probably due to the greater fatness of sheep shown during this period (see Table XIX). The Southdown has decreased in weight in Period II but increased again in Period III. No breed has shown a constant decrease in weight at this age.

Table XVIII. *Changes in weight of sheep shown at Smithfield from 1893 to 1913.*

Prepared from Table I by correcting for age differences. Period I, 1893-1899; Period II, 1900-1906; Period III, 1907-1913. Weights in lbs. and decimals of a lb.

BREED	9 MONTHS OLD						21 MONTHS OLD					
	Period I		Period II		Period III		Period I		Period II		Period III	
	No. of sheep	Wt.	No. of sheep	Wt.	No. of sheep	Wt.	No. of sheep	Wt.	No. of sheep	Wt.	No. of sheep	Wt.
Leicester	81	163.2	84	178.2	81	175.8	99	270.4	84	286.4	84	288.3
Lincoln	60	194.5	78	197.0	63	200.5	78	320.0	81	337.5	63	319.9
Kent	81	166.2	168	172.2	108	165.5	108	269.7	162	271.7	91	270.7
Devon Longwool	81	171.0	51	184.0	24	199.0	63	261.6	18	265.2	15	226.0
Cheviot	30	153.4	57	165.4	87	164.4	159	203.5	63	237.9	84	228.3
Southdown	348	137.4	291	140.4	324	130.5	246	202.8	204	195.8	210	200.2
Hampshire	318	179.4	228	176.5	198	172.6	177	276.3	162	277.3	90	268.6
Suffolk	114	183.6	114	185.6	153	188.4	78	277.6	78	289.4	63	275.6
Shropshire	99	151.6	102	159.0	72	160.0	165	236.4	75	254.0	99	244.4
Oxford Down	114	172.5	90	173.6	99	183.6	84	262.3	66	274.3	105	288.6
Average	10	167.3	10	173.2	10	174.0	10	258.1	10	268.9	10	260.5

The general conclusion arrived at from the average of all breeds shown at the bottom of Table XVIII is that at 9 months old the average weight has steadily increased from 1893 to 1913, but that at 21 months old it has remained practically constant only showing a temporary rise in the second period, due probably (as Table XX shows) to the sheep being more heavily fattened during this time.

Table XIX. *Comparative carcase weights of sheep of 21 months old.*

Average weights of sheep shown at Smithfield in 1840-2 and in 1893-1913.

BREED	PERIOD	CLASS	No.	Carcase weight, lbs.	Fat lbs.
Leicester	1840-42	—	72	131.2	16.7*
	1893-1913	Carcase	2	130.5	9.1
	1893-1913	Live†	267	186.5	—
Southdown	1840-42	—	37	122.6	17.5‡
	1893-1913	Carcase	57	90.8	7.8
	1893-1913	Live§	660	132.4	—

\* Average of three individuals.

‡ Average of four individuals.

† Estimated as 65.2 % of live weight.

§ Estimated as 65.8 % of live weight.

As the period 1893-1913 covers only a small space in the history of breed improvement, the weights of sheep shown at Smithfield in 1840-2 are given in Table XIX for purposes of comparison. From this table it will be seen that Leicester sheep exhibited in the Carcase Classes of to-day are approximately the same size as those shown 70 years ago, but the sheep shown in the Live Classes of to-day are some 55 lbs. per carcase heavier. Southdown sheep exhibited in the present day Carcase Classes are much lighter and those exhibited in the Live Classes much heavier than those shown in 1840.

The significance of these changes is realised when the weights of fat are compared, the sheep of 1840 being much fatter than those of the Carcase Classes of to-day and probably correspond to those of the present day Live Classes<sup>1</sup>. It is evident therefore that since 1840 the Leicester has increased considerably in weight while the Southdown has probably increased to a small extent only.

Table XX shows how the proportions of the body in four breeds of sheep—Cheviot, Hampshire, Suffolk and Southdown—have varied during the period 1893-1913.

Table XX. *Changes in the proportional development of sheep from 1893 to 1913.*  
Given as percentage of live weight. Period I, 1893-1899; Period II, 1900-1906; Period III, 1907-1913.

BREED	PERIOD	9 MONTHS OLD					21 MONTHS OLD					"Une counted for"
		No. of sheep	Carcase	Fat	Ploek	Skin	No. of sheep	Carcase	Fat	Ploek	Skin	
Cheviot	I	2	61.16	5.34	2.91	12.13	17	66.69	6.30	2.82	8.18	15.92
	II	14	61.65	5.78	2.98	10.49	12	65.59	6.48	2.76	10.05	15.12
	III	51	59.06	5.35	3.25	11.74	47	61.16	6.11	2.98	10.25	19.50
Hampshire	I	26	63.83	5.26	3.12	8.68	13	67.02	5.53	2.68	7.26	17.51
	II	33	62.66	5.83	3.14	9.82	15	67.74	6.92	3.16	8.23	13.95
	III	25	61.05	5.10	3.36	10.63	7	62.37	5.89	3.50	8.65	19.59
Suffolk	I	19	64.88	6.25	3.15	8.32	6	68.83	6.44	2.58	6.83	15.32
	II	64	61.95	6.35	3.27	8.90	20	64.73	6.36	3.09	7.63	18.19
	III	71	60.50	5.98	3.27	9.75	35	63.01	6.99	3.07	7.84	19.09
Southdown	I	17	63.67	5.48	3.29	8.18	8	69.15	4.95	2.56	7.79	15.55
	II	29	63.40	5.36	3.15	8.93	19	66.34	5.90	2.88	8.50	16.38
	III	34	61.08	5.78	3.56	10.50	30	62.65	5.59	3.07	8.82	19.87
Average of four breeds	I	64	63.70	5.61	3.17	8.55	44	67.54	5.88	2.70	7.65	16.23
	II	140	62.39	5.96	3.18	9.28	66	66.03	6.38	2.98	8.46	19.15
	III	181	60.27	5.64	3.34	10.37	119	62.18	6.22	3.06	9.09	19.45

The average of the four breeds given at the bottom of the table shows very strikingly that the carcase percentage has steadily declined

<sup>1</sup> I have weighed the internal fat of four Suffolk sheep of 21 months old from the Live Classes of the 1920 Smithfield Show and found on the average 20 lbs. of internal fat; the average for the Carcase Classes being 12½ lbs.

from 1893 to 1913 by 3.5 per cent. at 9 months and 5.3 per cent. at 21 months old. This decrease is not associated with any change in the fatness of the animals for the percentage of fat at both ages rises in Period II and falls again in Period III. The fall in carcase percentage is associated however with a marked rise in the percentage of pluck, skin and alimentary canal ("Unaccounted for").

Each breed considered separately shows, with but slight variations, the same changes.

It is difficult to find an explanation for these changes. It would appear that the sheep of to-day are not so well fleshed although quite as fat as formerly, this being brought about possibly by changes in the systems of feeding and management as the effects of exercise and protein food are believed to affect muscular development.

**Season.** In some years sheep grow and fatten better than in others, that is in addition to the other causes of variation in weight there is variation due to season. Table XXI shows how the sheep of five different breeds vary each year from the average weight of the whole period. In the table the sheep of 21 months old have been given in the year in which they were born, and it will be noticed that if this is done there is more similarity between the two curves than if they were given in the year in which they were exhibited. The coefficient of correlation has been calculated between the seasonal variation in weight of sheep of 9 months with that of sheep of 21 months exhibited the following year and is  $+ .57 \pm .012$ . This seems to show that the influences which affect the animal in the first year of life are those which are most important in influencing its size.

The problem of whether a check on growth in the early stages is compensated for later in life and whether a "Store" period affects the ultimate size of an animal is one in which much conflicting evidence exists. Hopkins<sup>(50)</sup> found in rats that growth stopped by deficiencies of diet could be made up at a later stage. Hatai<sup>(51)</sup> found in rats that the reduction in weight due to three weeks partial starvation was completely compensated for by subsequent accelerated growth. Osborne and Mendel<sup>(52)</sup> too found that in rats the ability to grow is not lost with age provided growth has not functioned during the period usually associated with increase in size. Osborne *et al.*<sup>(53)</sup> have shown that accelerated growth takes place in rats after a period of suppression due to an inadequate ration. Morgulis<sup>(54)</sup> however states that periodic starvation is more detrimental to the animal than acute starvation followed by a liberal supply of food. Watson and Hunter<sup>(55)</sup> found that in rats a

permanent stunting of growth was associated with the use of a faulty diet in the growing period, while Henseler<sup>(56)</sup> has shown that this is true in pigs. Jackson and Stewart<sup>(57)</sup> found that rats starved when young failed to reach the adult size of controls from the same litter. Waters<sup>(58)</sup> found that young cattle kept on maintenance rations for a year remained stationary in weight but that the skeleton grew at the expense of the muscles; in animals kept on sub-maintenance rations the skeleton also grew although the animals lost weight. These results have

Table XXI. *Effect of season on rate of growth in sheep.*

Total of five breeds: Leicester, Southdown, Suffolk, Hampshire, Cheviot. Compared with the variations in rainfall and root and hay crops. For explanation, see p. 374.

YEAR	TOTAL OF FIVE BREEDS		RAINFALL Inches	TURNIP CROP Tons per acre	HAY CROPS Cwts. per acre
	9 months	21 months*			
1892	—	-21	- 2.97	+1.22	+ 2.93
1893	-29	-31	- 5.11	-0.46	-11.64
1894	+23	-35	+ 2.82	+0.98	+ 4.79
1895	-29	- 7	- 2.00	-0.42	- 2.42
1896	-69	-39	- 2.38	-1.58	- 5.48
1897	- 4	0	+ 0.82	+0.74	+ 1.05
1898	-22	-50	- 3.90	-1.96	+ 5.95
1899	-27	+ 1	- 1.80	-3.31	- 0.77
1900	+15	+59	+ 3.05	+1.12	+ 0.46
1901	+ 3	+34	- 3.49	-1.06	- 5.49
1902	+19	+51	- 5.31	+2.13	+ 4.28
1903	+23	+62	+10.49	-0.57	+ 3.01
1904	+34	+ 3	+ 3.07	+0.51	+ 1.40
1905	0	+28	- 4.52	+0.29	- 0.86
1906	- 3	- 3	+ 0.91	+0.49	- 0.87
1907	+ 3	+ 2	+ 0.29	+1.32	+ 4.50
1908	-22	+10	- 2.67	+1.25	+ 2.61
1909	-15	-15	+ 1.98	+3.11	- 0.25
1910	-16	+11	+ 5.21	+2.99	+ 2.50
1911	-20	+14	- 0.72	-3.81	- 4.84
1912	+13	+41	+ 8.53	0.64	- 0.37
1913	+36	—	+ 0.96	-0.51	+ 2.74

\* Year in which born, not in which exhibited.

been confirmed by Trowbridge, Moulton and Haigh<sup>(59)</sup>. Aron<sup>(60)</sup>, as a result of experiments with dogs and observations on children, concludes that growth primarily depends on the tendency to enlarge possessed by the skeleton and that the skeleton loses its capacity to grow in more advanced age, regardless of the size which the animal has reached. He found that an animal which had been kept for a period on a limited diet never reached the ultimate size of one which had been normally fed.

Table XXI shows the yearly variation in the weight of sheep with the yearly variation in rainfall and it will be seen that speaking generally a large rainfall is associated with high live weight. The coefficient of

correlation has been calculated that between sheep of 9 months and rainfall is  $+ .35 \pm .13$  and that between sheep of 21 months and rainfall is  $+ .38 \pm .13$ . The breeds—Leicester, Southdown, Suffolk, Hampshire and Cheviot—are fairly representative of all conditions found in Great Britain and the average rainfall for England and Wales has been compared with the total for these breeds; possibly a local breed compared with local rainfall in each month of the year would show closer approximation.

The way in which the rainfall influences live weight is probably through its effect on the root and forage crops; in a dry year with a shortage of these crops the lambs receiving a check in growth. The yearly variations in the turnip and hay crops are shown in the last two columns of Table XXI.

#### SUMMARY.

Records of the live and carcase weights of sheep exhibited at the Smithfield Show from 1893 to 1913 have been treated statistically.

The results show the average weights, rates of growth, and proportions of the carcase in the different breeds of sheep at 9 months and at 21 months of age (see Tables II and V).

On the average of all breeds the rate of growth declines from 4.7 lbs. per week from birth to 9 months of age to 1.7 lbs. per week from 9 to 21 months of age.

The carcase percentage increases with age on the average from 61 per cent. at 9 months to 65 per cent. at 21 months and with it the percentage of fat from 5.7 per cent. at 9 months to 6.4 per cent. at 21 months. On the other hand the proportions of pluck, skin and alimentary canal ("Unaccounted for") decrease with age.

Ratios of early maturity are given for the various breeds and the factors which affect it are discussed.

The average weights, rates of growth and proportions of the carcase are given for the different crosses of sheep (see Tables IX and XIII).

From a comparison of crossbreds with pure breeds it would appear that crossing leads to increase in live weight and probably more early maturity. There is an indication that the proportions of carcase, fat and pluck are less but the proportions of skin and alimentary canal are greater in crossbreds than in the pure breeds of sheep.

There is greater variation in live weight in the Cheviot and Blackface breeds than in the Leicester, Southdown, Hampshire and Suffolk breeds;

in the latter breeds variability decreases but in the former breeds it increases with age.

Variability in live weight at 9 months old has steadily increased from 1893 to 1913 but at 21 months old there has been little change.

Some parts are more variable in their proportion to live weight than are others. Fat and alimentary canal are most variable, skin and pluck slightly less variable, while the proportion of carcase is less variable even than live weight.

The variability of the proportions of carcase, pluck and alimentary canal increase while the variability of the proportions of fat and skin decrease with age.

Within a breed and among animals of the same age the heaviest sheep generally have the highest carcase and fat percentage and the lowest percentage of pluck, skin and alimentary canal.

A high proportion of carcase is correlated with a high proportion of fat and a low proportion of skin and alimentary canal at 9 months old. At 21 months old the same holds true with the exception that the sheep with the highest carcase percentage have not the most fat.

The proportion of pluck does not appear to be correlated with any other part of the body except inversely with the live weight.

During the period 1893-1913, although individual breeds show differences, the majority have increased in live weight at 9 months old but at 21 months old have remained practically constant in weight.

The sheep exhibited in 1840 were very much fatter than those shown in the Carcase Classes of to-day and are probably comparable with those of the present-day Live Classes. Since 1840 Leicesters have shown a great increase and Southdowns a small increase in weight.

The carcase percentage of sheep both at 9 and 21 months of age has steadily decreased during the period 1893-1913; this has been attended by an increase in the proportion of pluck, skin and alimentary canal.

There is an indication that the influences which affect sheep in their first year of life (store period) affect their ultimate size.

The seasonal variation in the weight attained by sheep is dependent on the rainfall; a high rainfall through increased root and fodder crops causing increased live weight.

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## A CALORIMETER FOR USE WITH LARGE ANIMALS.

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(With Five Text-figures.)

IN recent years the measurement of the heat output of animals has taken a prominent place in investigations on the physiology of nutrition, and especially so as regards farm animals. As it is now accepted that the conservation of energy applies to the chemical changes in the living body, the total metabolism can be measured equally well by the indirect or by the direct method. The indirect method using the Douglas bag has been shown by Benedict and others to be simple and satisfactory in the case of human beings, but it is not so easily applied to farm animals, and for these the direct method by the use of a calorimeter has advantages.

In the case of most biological measurements of heat output great accuracy is not needed. Since two similar animals in apparently similar circumstances, or the same animal at different times, may differ in their heat evolution by 20 per cent. or more, it is clearly superfluous to introduce great complications in the calorimeter, entailing great expense and a considerable staff of observers, to secure minute accuracy. For some purposes the highest possible accuracy *is* necessary, as for instance in the experiments establishing the conservation of energy in vital processes. For the bulk of the experiments on nutrition however it is much more useful to get a mean result from a large number of different observations, even though the possible error of a single experiment may reach two or three per cent. Systematic errors can be avoided. They arise merely from insufficient investigation of the conditions of the experiments, or inaccurate calibration of the various measuring appliances.

Such calorimeters of large size as have been hitherto erected have been in almost every case designed on the assumption that leakage of heat must be prevented. The adiabatic calorimeter of the Atwater and Rosa type no doubt achieves this to a great extent, but at the cost of great expense and complication. Is the elimination of leakage really necessary? Leakage obeys a definite and simple law. If the circumstances

are known with sufficient accuracy the leakage can be calculated. The ordinary bomb calorimeter is by no means free from leakage, yet it gives consistent and accurate results.

The calorimeter described below is the result of an attempt to devise an instrument that is simple in operation, sufficiently accurate for most biological measurements, and capable of being erected at a cost that is within the means of any Agricultural College.

It is difficult to say what it would cost to instal such a calorimeter at the present time. Much of the present instrument was set up before the War when prices were lower, and many experimental changes have been made in the course of its development, so that the actual cost is no guide. If a suitable room were available and no reconstruction were needed, the calorimeter could probably be set up with all its subsidiary appliances for not more than £500.

The carcass of the calorimeter was erected in 1914 by Professor A. V. Hill on the general lines of several smaller instruments described by him. A brief account of the calorimeter was published by him in the *Journal of Physiology*, 58, with a few preliminary test measurements.

Nothing further was done with it during the war.

In the summer of 1919 the present writer took charge of the apparatus. He has made some changes in the original construction of the body, and is wholly responsible for the subsidiary appliances.

#### *The Calorimeter Room.*

The room in which the calorimeter is placed is in the basement on the north side of the School of Agriculture, half the height of the room being below the ground level. As it is never touched by the sun its temperature varies very little in average weather. The temperature is recorded continuously by a Richard thermograph and it is found that a change of as much as  $1^{\circ}\text{C}$ . in the 24 hours is rarely exceeded. A sudden severe frost or a rapid and great rise of temperature has occasionally caused difficulties. These difficulties could be avoided by regulating the temperature of the room by a thermostat. An adjustable electric heater is sometimes used for bringing the temperature of the room approximately to that of the inside of the calorimeter in order to reduce the leakage of heat, but regulation by a thermostat has not hitherto proved to be necessary.

The room is 33 ft. by 15 ft. and 8 ft. high. The calorimeter stands in the middle of the room, the outer casing reaching from floor to ceiling. It would have been preferable to have an air space above and below but

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the dimensions of the room did not permit this. The floor area of the calorimeter and casing is 11 ft. by 8 ft.

The temperature of the room is kept uniform in all parts by means of a fan which causes a circulation of air round the casing and, being directed slightly upwards, prevents the formation of a layer of warm air near the ceiling.

### *The Body of the Calorimeter.*

The body consists of a cylindrical galvanised iron tank 9 ft. 6 ins. long and 5 ft. 6 ins. in diameter, lying on its side on four substantial wooden supports, which raise the bottom of the tank about a foot from the floor.



Fig. 1.

The plates of which the tank is made are  $\frac{1}{16}$  in. thick. A  $\frac{3}{4}$  in. lead pipe is soldered in a helix round the outside of the cylindrical part of the tank, and in a spiral over one end, the separate turns being about 5 ins. apart. The door covers the other end and has no pipe fixed to it.

Fig. 1 is a photograph of the tank before it was cased in and insulated.

Water maintained at a constant temperature by a thermostat circulates through the lead pipe, and finally through 80 ft. of half-inch gilled tube of the kind used for motor car radiators suspended near the roof of the tank.

The quantity of heat given off by an animal is calculated in the usual

way from the rate of flow of the water and the difference of temperature between the inlet and outlet water, with the corrections to be described later.

When the calorimeter was first erected, it was insulated with sawdust and wood turnings. This answered its purpose for a time, but after it had been standing during the four years of the war, it was found to be heating through the growth of a fungus, and it was therefore taken out and replaced by granulated cork.

As the floor of the room is concrete resting on the bare earth the insulating material would in course of time become damp if allowed to rest on the floor, even if there were none of the casual floods to which the basement of a building is liable. Hence a corrugated iron floor about 2 ins. above the concrete floor is placed under the calorimeter. The whole is enclosed in a wood casing of  $\frac{1}{2}$  in. tongued and grooved boarding and the space between the casing and the tank is filled in with granulated cork, nearly a ton being required for the purpose. Finally the whole outside of the casing is papered to check any circulation of air through the joints in the boarding. If the boarding is not exceptionally tight this papering is quite necessary. It was found by experiment to reduce the leakage appreciably.

The tank is nowhere covered with less than 10 ins. of cork, and in most parts there is a greater thickness.

The door covers the whole of one end of the tank. In its original form it was found to allow heat to pass much too readily. It has therefore had successive layers of insulation added and is a somewhat heterogeneous assembly. The leakage through the door is now not great but it could probably be reduced still more. The door as at present constructed has on the inside a sheet of bright tin followed by 2 ins. Kapok wool, 1 in. wood, 3 ins. sawdust,  $\frac{1}{2}$  in. tongued and grooved boards, and outside all a layer of thick felt covered with oil-cloth. It has a small double window for observation of the animals, the window being covered with felt when not in use.

A 10 c.p. electric lamp is fixed in the calorimeter for use when required. As experiments may sometimes be made with human beings there is also an electric bell, which can be rung from inside, and a speaking tube.

The door end of the tank is fitted into a heavy wood framing and the joint made airtight by putty and paint. The door is hinged to this framing and is pressed against strips of india-rubber by eight clamps distributed round its sides.

*The Water Supply.*

The water which circulates round the calorimeter is supplied from a tank holding about 200 litres and situated some 20 ft. above the calorimeter. This leads to difficulties in the insulation of the supply pipe, which is 80 ft. long and has to pass through several rooms—an arrangement which could not be avoided in the present circumstances. It would be much better to have the supply tank in the calorimeter room if there were sufficient height. The Cambridge water is highly aerated, with the result that air-locks form in the pipes if a considerable pressure is not maintained in them. The avoidance of air-locks will be referred to again in a later section.

The circulating water is maintained at a constant temperature by an electrical thermostat of the usual pattern immersed in the supply tank. The expansion chamber of the thermostat is 20 ft. of half-inch lead tube coiled in a spiral and filled with ordinary paraffin oil. Sealed to this is a glass U tube containing mercury whose rise and fall closes or opens an electric circuit.

It is a matter of little consequence what liquid is chosen for the expansion liquid, as all the liquids in general use such as xylol, aniline, toluol, etc., have nearly the same coefficient of expansion. Paraffin was chosen because it allows of a very simple and efficient method of sealing the glass tube to the lead tube. Shellac melted with 20 per cent. of oil of cassia makes an excellent cement for the purpose. It is quite unaffected by paraffin, makes a tight joint, is not brittle, and melts at such a moderate temperature that there is little risk of cracking the glass.

The dimensions of the thermostat are such that a rise of 5 cms. of the mercury corresponds to 1° C.

The current which is opened and closed by the mercury is given by eight dry cells, and passes through a relay of the Post Office pattern of about 2000 ohms resistance. Soiling of the mercury surface seldom gives any trouble.

The heating circuit worked by the relay contains two 50 c.p. carbon filament lamps immersed in the water. The lamp connexions are kept dry by motor tyre tubes stretched tightly over the lamp bulbs. A little waterproof varnish poured round the inside of the joint where the rubber touches the glass is a useful precaution, as without it the water is found to creep through in course of time and cut out the lamp.

The heating circuit cannot be opened and closed by the platinum contacts provided on the relay, as the current is too great. In general

about 2 amps. is used and this soon burns the contacts. A light wood arm about a foot long is therefore attached to the armature of the relay and bears on its outer end an inverted U-shaped piece of stout platinum wire which makes contact between two mercury cups forming part of the heating circuit. One leg of the U wire is long enough to make permanent contact with its mercury cup. The other leg makes and breaks contact according as the mercury in the paraffin thermometer opens or closes the subsidiary circuit in the relay. The mercury in the cup is burnt slowly by the spark and needs cleaning and replenishing occasionally. This can be done without interference with the working of the thermostat.

In some of the experiments the circulating water has to be kept at from  $25^{\circ}\text{C}$ . to  $30^{\circ}\text{C}$ . This needs a greater current than can be sent through the relay, so in such cases the relay current is used merely as a fine adjustment, the greater part of the heating being done by heaters which are kept permanently in circuit. For this purpose spirals of Ni-Cr wire wound on fire clay bars as supplied for domestic heating are used. Five bars are placed in a thin copper box immersed in the water, the terminals being so arranged that any number of bars from one to five can be used according to the temperature required. The bars are nominally 500 watts each, hence each gives about 7 large cals. per min. Thus if the temperature of the water supply and the rate of flow of the water are known, the number of bars to be used to maintain any given temperature can readily be found.

The water in the supply tank is kept vigorously stirred by a fan driven by an electric motor. It is necessary to secure that every part of the water in the tank is in motion.

The thermostat has been found to be remarkably efficient. The water in the tank can be maintained for long periods at a temperature which does not vary by as much as  $0.1^{\circ}\text{C}$ . Unfortunately the steadiness of temperature is not quite so great when the water reaches the calorimeter. The supply pipe is cased throughout its length and the casing filled with insulating material, yet there is a slight rise of temperature during the day and a slight fall during the night. This change rarely exceeds  $0.2^{\circ}\text{C}$ . in the course of 24 hours and is so slow that it has no appreciable effect on the results.

Before the water starts on its circulation round the calorimeter it passes through a metal vessel shaped as shown in Fig. 2. The water enters at A

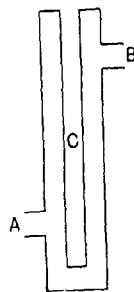


Fig. 2.

and leaves at *B*. The chamber *C* is filled with water, and has a Beckmann thermometer placed in it to record any changes in the temperature of the water supply. An occasional glance at the thermometer shows whether the thermostat is working properly or not.

The metal chamber is fixed on the outside of the calorimeter casing, and is itself cased and insulated.

A similar chamber is placed in the outlet for purposes to be explained later.

The water flow is determined by diverting the stream from the outlet for a few minutes into a large vessel and weighing the contents.

The flow is in general about 2 litres per min. and can be adjusted by a tap at the outlet. This adjustment is a convenient way of regulating the rise of temperature of the water for any given evolution of heat.

#### *The Temperature Measurements.*

The difference of temperature between the inlet and outlet water is measured by a thermoelectric couple, the electric current produced by the couple being recorded continuously on a thread recorder made by the Cambridge and Paul Instrument Co.

The inlet and outlet ends of the pipe carrying the circulating water are brought near each other, and on each is fixed a metal chamber similar to that shown in Fig. 1. The two sets of junctions are placed respectively in the two inner chambers and the ends plugged with cotton wool. An attempt was made to place the junctions actually in the circulating water, but this was abandoned, as it was found difficult to seal the point of entry of the wires so as to stand the head of water, and the insulation of the wires was found to be liable in course of time to break down and cause an electrolytic current. Moreover it was found to be unnecessary. The arrangement adopted, where the wires are in an air space separated from the water by thin copper, proves to be more prompt in its indications than any other part of the apparatus.

The thermoelectric couple has 15 copper-constantan junctions. The wires after being soldered are dipped in melted paraffin wax, and the ends that had been bared for soldering are covered each with a sheath of thin india-rubber tube. The whole bundle of wires is then bound together with string and slipped into the metal chamber.

The couple should be made from wire of a gauge that will keep the resistance fairly low, as a high resistance lowers the sensitiveness of the galvanometer. The couple in use at present is made from No. 25 copper wire and No. 26 constantan and has a resistance of about 7 ohms.

A resistance box is placed in the circuit in order to bring the deflection within the range of the scale of the recording galvanometer when necessary. The total range of the scale of the galvanometer is 80 mm., and this corresponds to a little under  $1^{\circ}$  C. with the present arrangements.

For accuracy of reading it is desirable that the deflection should be fairly large, and it is not desirable that the actual difference of temperature measured should be large, for this, as will be seen later, causes uncertainty in the calculation of the leakage correction.

In general the temperature difference aimed at is about  $.75^{\circ}$  C. The temperature difference is readily adjusted to about this amount for any given rate of evolution of heat by adjustment of the water flow.

The method of finding the deflection per degree difference of temperature is described in a later section.

For calibration and test purposes a constantan resistance wire is stretched on a wood frame on the upper part of the inside wall of the calorimeter, so high as to be out of the reach of animals. The total resistance of the wire is 63.3 ohms. A definite amount of heat can thus be introduced into the calorimeter by passing a measured current through the wire. As it seemed likely that the resistance of the wire would depend to some slight extent on the strength of the current, the original intention was to measure both the current in the wire and the electromotive force between its terminals, and calculate the heat introduced from the expression  $\frac{CE \cdot t}{4.18}$ , which gives the small calories evolved in  $t$  seconds. It proved however that the constantan wire used has no appreciable temperature coefficient. A series of 18 determinations was made of the resistance by dividing the E.M.F. by the current, the current strength ranging from .6 amp. to 3.1 amps. The extreme range of the readings was from 63.0 to 63.5 ohms. with no indication of progressive variation. The mean was 63.3 ohms. The resistance measured with a Wheatstone bridge at the temperature of the room was 63.29 ohms.

A further test was made by measuring the resistance of a sample of the same wire at  $0^{\circ}$  C. and  $100^{\circ}$  C. The change in resistance was quite inappreciable.

This constancy in the resistance obviated the need for taking readings of the voltmeter. The heat introduced by the current is therefore calculated as  $\frac{C^2 R}{4.18}$  small cals. per sec.

Current and electromotive force are measured by two Weston millivoltmeters, one of which has an external shunt to convert it into an



ammeter, and the other a high external resistance to bring its scale readings within the range of E.M.F. in use. Both were calibrated by comparison with the standard instruments in the Cavendish Laboratory.

*Ventilation.*

The air required for ventilation enters the calorimeter by a 2 in. pipe near the top of the door, and leaves by a similar pipe near the centre of the other end of the calorimeter. It is drawn through the exit pipe by a motor driven fan. To measure the volume of air supplied a box 2 ft. long and 1 sq. ft. in section is placed between the exit pipe and the fan. This box is divided in the centre by a diaphragm of tinplate pierced with a circular hole an inch in diameter. The volume of air passing can be calculated from the area of the hole and the difference of pressure on the two sides of the diaphragm. This method of measuring air flow has been investigated by Professor W. Watson who finds that it is capable of giving results accurate within one part in 300. Details of the method with the precautions necessary for securing accuracy and the method of calculation are given in his paper in the *Proceedings of the Institution of Mechanical Engineers* for May 1912.

The air flow in cubic centimetres per second is given by the expression

$$V = \alpha F \sqrt{\frac{2gp}{\rho}},$$

where  $F$  = area of the hole in sq. cm.

$p$  = difference of pressure between the two sides of the diaphragm in cm. of water.

$\rho$  = density of the air in grams per c.c.

The coefficient  $\alpha$  depends on the shape of the stream lines. It is not strictly constant, but varies a little with variations of  $F$  and  $p$ . It may however be regarded as constant if the variations of  $F$  and  $p$  are not great.

Watson gives its value for various circumstances, but, as it is often difficult and in the case of the present apparatus impracticable to reproduce all the conditions, a direct measurement was made of the coefficient.

The thermostat was set to maintain the interior of the calorimeter considerably above the temperature of the room and the ventilating fan was kept running until the galvanometer deflection was steady and the hygrometric state of the interior constant, as was shown by the identity of the water content of the entering and leaving air.

The circulating water left the calorimeter cooler than it entered, and

the corresponding loss of heat was accounted for by leakage and by the heat carried out by the ventilating air. As the specific heat and density of the extracted air can be calculated from its temperature and hygrometric state and the height of the barometer, the leakage by a method described later, and the fall of temperature of the air in passing through the calorimeter is observed, the volume of air passing can be calculated and hence the coefficient  $\alpha$ .

Four determinations were made and gave the values

.629      .623      .629      .632

In the calibrations and tests described later the fan used for ventilation gave a difference of pressure of rather less than half an inch of water, which corresponds to an air flow of something over 200 litres per min. the actual flow depending on the temperature, etc., of the air. Such a small difference of pressure cannot be read with sufficient accuracy, and 200 litres per min. is an inadequate air supply for any but small animals. Consequently a Keith-Blackman centrifugal fan driven by a series wound motor has now been installed. This will give a difference of pressure of 6 ins. of water with a hole an inch in diameter. By varying the speed of the fan by introducing resistances in the motor circuit and by using holes of various sizes the air flow can be adjusted over a very wide range. The constant for this new fan when the air flow is from 600 to 700 litres per minute has been found to be .594.

#### *Hygrometry.*

As a considerable proportion of the heat to be measured leaves the calorimeter with the ventilating air in the form of the latent heat of water vapour it is necessary to know the water content of the entering and leaving air. The principle of the Assmann ventilated psychrometer has been adopted for the determination of the water vapour. If air passes over dry and wet bulb thermometers with a velocity not less than three metres per second the humidity of the air can be calculated from the readings of the thermometers with an accuracy little if at all inferior to that obtained by the chemical method. Tables giving the dew point or the vapour pressure obtained from the readings of the ventilated psychrometer are given in several books of tables, such as the Smithsonian Physical Tables, Landolt and Börnstein's Tables and others.

The thermometers are placed in two cubical boxes of 3 in. side, one of the boxes being in the inlet pipe and the other in the outlet. It is not practicable to calculate the air velocity from the total flow and the dimensions of the box, as the air probably passes through in a more or less

definite jet. In order therefore to find whether the minimum velocity had been reached, the apparatus was tested against a standard Assmann psychrometer kindly lent by Sir Napier Shaw, the Director of the Meteorological Office. Many comparisons were made and it was found that the dew point of the air calculated from the calorimeter readings never differed by more than one or two tenths of a degree from that found by the standard psychrometer.

One warning should be given here. The wet bulb must be wetted only with distilled water. If the town supply is used there will soon be such an accumulation of soluble salts in the muslin as will make the readings too high from the reduction of the vapour pressure.

The dry bulbs at the inlet and outlet serve for finding the temperature of the entering and leaving air. A correction has to be made for the heat introduced or removed by this air. The thermometers now in use have a range of  $0^{\circ}$  C. to  $30^{\circ}$  C. They are graduated to fifths of a degree and their errors have been determined at the National Physical Laboratory.

#### *Calibrations and Tests.*

Perhaps the most important calibration and certainly the most difficult is the conversion of the galvanometer deflections to centigrade degrees, as it depends on the readings of a sensitive mercurial thermometer—an instrument which is far from easy to use.

It may not be superfluous to mention that a delicate thermometer is quite unreliable in a falling temperature, when hundredths of a degree are of importance. The mercury falls intermittently and even though the thermometer is tapped the final reading may be one or two hundredths above the actual temperature. The mercury must always *rise* to its final reading. This can be secured by a preliminary cooling of the thermometer when necessary.

Moreover if a thermometer has been used at, say,  $30^{\circ}$  C. and is then brought to a temperature  $15^{\circ}$  or  $20^{\circ}$  lower, the zero will change quite appreciably for some hours.

It has been stated above that double walled boxes are interposed in the inlet and outlet water pipes close to the similar boxes in which the thermoelectric junctions are placed. These boxes are used for containing the thermometers by which the galvanometer deflections are converted to centigrade degrees.

The standard thermometer used is an excellent instrument graduated in hundredths of a degree. It was calibrated at the Reichs Anstalt with

the bulb immersed to the zero line and the projecting thread at  $18^{\circ}\text{C}$ . These conditions were adhered to in the use of the thermometer.

The procedure was as follows. The thermostat was set to bring the inlet water near the room temperature in order to reduce any error due to leakage of heat into or out of the thermometer boxes. A current was then started in the resistance wire of such a magnitude as to give a convenient rise of temperature. This current was left running for several hours until the steadiness of the galvanometer deflection showed that the outlet temperature was steady.

The thermometer was then cooled a little, placed in the inlet water box and left to rise until it was steady. This took about ten minutes. It was then removed to the outlet box and left for ten minutes, cooled and removed to the inlet box, and so on until there were three readings of the inlet temperature and two of the outlet. The mean of the three inlet readings subtracted from the mean of the two outlet readings gives the difference of temperature and a simultaneous reading of the galvanometer deflection gives the number of millimetres deflection per  $1^{\circ}\text{C}$ .

This procedure was repeated with various differences of temperature.

The scale of the galvanometer extends only to 80 mm. If the temperature difference would give more than this, the deflection can be reduced to a convenient value by putting resistance in the galvanometer circuit. The calculation of the millimetres per degree in this case requires a knowledge of the actual resistance of the galvanometer circuit. The resistance can be measured by a Wheatstone bridge provided there is no thermoelectric E.M.F. in the circuit. This is known to be the case when there is no deflection shown by the galvanometer, but a safer method is to take the junctions out of the calorimeter and put them in a padded box or a large thermos flask for an hour or two before the resistance is measured.

In the present apparatus the galvanometer, thermo-couple and leads have a resistance of 14.55 ohms and the final mean calibration of the deflection is 92.25 mm. per  $1^{\circ}\text{C}$ .

The proportionality of deflection to current was tested by varying the resistance of the galvanometer circuit when the temperature was steady. The deflection was found to be inversely proportional to the total resistance within the limits of the error of reading.

The range of temperatures measured is so small that no error arises from variation in the thermoelectric power of the junctions with temperature.

It appeared at an early stage that the leakage of heat was quite considerable. This proved to be largely through the door, which was less

adequately insulated than the rest of the walls. Layers of additional insulation were added as mentioned above by which the leakage was reduced to a more reasonable amount, but there is still some room for improvement in the door.

The leakage constant was determined in the following way:

The thermostat was set to some temperature different from that of the room and the apparatus left running for some hours. The deflection gradually approached zero, and in the test cases where the thermostat had been set to the room temperature it settled down to zero and remained there. When the inlet water was warmer than the room there was a leakage outwards and the outlet water was cooler than the inlet, as was shown by a negative deflection of the galvanometer. This deflection became steady after a time which varied with the initial circumstances. It was necessary to wait until the temperature gradient in the cork had become steady, and this may take several hours.

The deflection in mm. divided by 92.25 gives the difference of temperature between the outlet and inlet water, and this multiplied by the water flow gives the loss of heat by leakage. Dividing this again by the difference of temperature between the inlet water and the air we get the leakage per degree difference of temperature.

The determination cannot be made with great accuracy as it depends on the measurement of galvanometer deflections of a very few millimetres. This is however of little consequence, since in ordinary use the leakage can be made small by adjusting the temperature of the room to that of the calorimeter.

The following table shows the degree of concordance obtained. The upper line gives the excess of temperature of the inside over the outside. In the two cases where a minus sign is prefixed the inside was colder than the outside and the leakage was therefore inwards. The lower line gives the resulting leakage in large calories per day per degree difference of temperature.

TABLE I.

Temp. diff.	10.3°	6.8°	5.7°	5.5°	5.4°	4.2°	3.0°	-1.5°	-2°
Leakage	119	113	118	121	111	108	118	121	118

The mean is 116 calories and this value was used in the succeeding tests.

In making these determinations it is of no consequence whether we take the inside temperature to be that of the inlet water or of the outlet water. The difference between the two is so small that it can be neglected compared with the difference between the inside and outside.

The next set of tests was made mainly to find how the leakage correc-

tion affected actual measurements simplified by the omission of ventilation air.

The two ventilation pipes were closed by large corks to prevent any circulation of air between the inside and outside, and a steady current was passed through the resistance inside the calorimeter until the galvanometer reading was steady.

The heat introduced electrically is calculated in large calories per minute from the expression  $\frac{60 C^2 R}{4.18 \times 1000}$ . The heat extracted by the circulating water is given by the water flow in litres per min. multiplied by the rise of temperature. If to this is added the leakage correction the total should be equal to the electrically introduced heat. Here however a difficulty presents itself. It is necessary to know the internal temperature to calculate the correction, and the temperature varies at different parts of the calorimeter. The important factor in the leakage is the temperature of the metal tank and this cannot be assumed to be at the same temperature as the inside air. Where the water enters, the tank will be at the temperature of the inlet water or nearly so, and where the water leaves, the tank will be at the temperature of the outflow, which may be a degree or more above the inflow. The effective temperature causing the leakage is between these two. The most obvious plan is to try using the mean. The fact that the water passes through the radiator tubes suspended near the roof immediately before leaving the calorimeter, and thus probably picks up an appreciable amount of heat after leaving the walls of the tank, makes it likely that the best value to take for the tank temperature is somewhat below the mean of the inlet and outlet. The measurements detailed below show that, if this over-correction exists, the error is small, as there is no indication of a progressive excess of extracted heat over introduced heat as the difference of temperature between the inside and outside increases.

TABLE II.

Difference of temperature	Uncorrected output	Leakage correction	Corrected output	Heat introduced	%
6.2°	3.143	.499	3.642	3.635	100.2
5.8°	2.312	.467	2.779	2.783	99.8
5.5°	1.608	.443	2.051	2.045	100.3
5.5°	1.807	.257	2.064	2.045	100.9
3.2°	1.809	.225	2.034	2.045	99.5
2.8°	1.809	.209	2.009	2.018	99.5
2.6°	1.800	.209	2.010	2.018	99.6
2.6°	1.801	.209			99.9
Mean	...	...	...	...	...

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The heat is in every case given in large calories per min. The first column gives the difference between the room temperature and the mean of the inflow and outflow temperatures.

The second column is the water flow multiplied by the rise of temperature of the water.

The third column is calculated from the first on the assumption that  $1^{\circ}$  C. difference of temperature gives a leak of 116 cal. per day.

The fourth column is the sum of the second and third.

The fifth column gives the heat introduced electrically, calculated from the current and the resistance.

The last column gives the extracted heat expressed as a percentage of the introduced heat.

In these tests the leakage was intentionally made large by raising the temperature of the interior of the calorimeter considerably above the air of the room, yet in spite of this the greatest deviation from the mean is only 1 per cent. In actual use the room temperature would be adjusted as nearly as practicable to the temperature of the interior of the calorimeter, hence it may be concluded that if ventilation air and water vapour are left out of account, the calorimeter is capable of giving results correct within 1 per cent.

It still remained to test the ventilation and hygrometry appliances. It is hardly practicable to test these separately. Some water producing liquid might be burnt or water evaporated in the calorimeter and the vapour extracted be determined by the wet and dry bulb thermometers, but the inner surface of the calorimeter is so great that a very protracted test would be necessary to bring this surface to a state of water equilibrium, and the result would always be open to doubt.

Consequently it was decided to make a comprehensive test of all the measurements and calibrations by burning alcohol in the calorimeter and determining its total heat of combustion. This again is not an entirely satisfactory test. If it be assumed that the sample of alcohol used contains only ethyl alcohol and water the proportions of the two can be determined from the specific gravity, but it is far from easy to secure that the alcohol used shall be quite free from other substances such as acetone, aldehyde, methyl alcohol, etc.

Moreover, the true heat of combustion of ethyl alcohol is not known accurately. Two sensibly different values are in common use—those of Favre and Silbermann and of Berthelot—and there seems little reason to prefer one to the other. The technical difficulties of making a direct measurement with the bomb calorimeter are so great in the

case of a volatile and hygroscopic liquid that this method is not suitable.

Nevertheless, as extreme accuracy cannot be expected in the present apparatus, it was decided that an alcohol combustion test would give a sufficiently good indication of the accuracy obtainable.

The alcohol was burnt in a small lamp constructed in the laboratory. A glass reservoir open to the air only by a fine capillary tube to avoid absorption of moisture supplied the alcohol to an ordinary spirit lamp burner. To secure complete combustion a chimney is needed. In the earlier attempts there was a marked smell of aldehyde in the calorimeter after the lamp had been burning for some time. Chimneys of various shapes and sizes were tried and the air supply varied until the lamp burnt without smell.

The float chamber of a motor carburettor was interposed between the reservoir and the burner to keep the level of the alcohol constant.

The rate of consumption increased in the first hour or two—presumably through the warming of the whole lamp. Afterwards the lamp generally burnt with great regularity, though occasionally there was found to be a progressive change in the rate. It was consequently necessary to have a check on the rate.

Before a test was made the calorimeter was warmed electrically to something near the temperature expected to be given by the lamp. The lamp which had been lighted for two or three hours was then weighed and placed in the calorimeter. Three or four hours later, when the galvanometer deflection was steady, the calorimeter was opened, the lamp taken out and weighed, and replaced. The disturbance of the curve on opening the door was quite temporary. Finally after some four or five hours more during which periodical readings were taken of air flow, wet and dry bulb thermometers, etc., the lamp was taken out and again weighed. If the rates of consumption in the two periods did not agree the experiment was rejected. Steadiness of the galvanometer deflection in itself gives a presumption in favour of regularity of consumption, but is not quite conclusive, as a change in the rate of consumption might be masked by a change in the state of water equilibrium of the inner surface of the calorimeter. It was therefore thought safer to make direct weighings.

The results of five combustion experiments are given below. All quantities of heat are in large calories per min. except the heat of combustion, which is in calories per gramme.

One experiment of the series was rejected because the galvanometer deflection did not get steady, and it was found that the alcohol lamp



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was burning irregularly. Another was spoiled by flooding of the float chamber.

TABLE III.

Heat extracted		TABLE III					
In circulating water	...	...	1.951	2.303	1.262	1.009	1.614
In water vapour	...	...	.159	.190	.177	.169	.100
In ventilating air	...	...	.024	.071	.116	.161	.000
Leakage	...	...	.048	.016	.112	.165	-.056
			2.182	2.580	1.667	1.504	1.658
Anhydrous alcohol burnt per min.			.3089	.3668	.2355	.2119	.2347
Therefore heat of combustion	...		7.061	7.033	7.078	7.098	7.065

The mean of the five determinations is 7.067 cal. per gram.

Berthelot's value is 7.068 Favre and Silbermann's is 7.183.

The Smithsonian Tables (7th Ed. Table 261) give 7.10 as the "mean from various observers."

It is not possible to state from these tests what is the probable error of a single observation, but it is clear that the accuracy is quite enough for most biological measurements.

### *Biological Tests.*

At the time of writing only a few biological tests have been made, and these mainly to test the proposed routine and to find the general character of the curves obtained. The results are of sufficient interest to be put on record, as they show the considerable and rapid variation in the metabolism of living animals, and the difficulties with which one has to contend in making measurements of such quantities as basal metabolism.

The first test was made with L. F. N. a member of the Staff of the Cambridge School of Agriculture. The test was merely quantitative.

Before L. F. N. entered the calorimeter a current of 1.3 amps. was run through the resistance in the interior to bring everything to a steady state. It was anticipated that this current would give approximately the same evolution of heat as L. F. N.

L. F. N. took a substantial lunch at 1 p.m. and tea and a few biscuits at 5 p.m. He took no other food before entering the calorimeter at 10 p.m., when he lay down on a camp bed and soon went to sleep.

The curve given by the recording galvanometer is shown in Fig. 3. The time is shown on the upper edge of the diagram. The ordinates are proportional to the E.M.F. in the thermoelectric circuit and therefore to the difference of temperature between the inlet and outlet water.

L. F. N. remained in the calorimeter for 12 hours during which time the temperature of the inlet water varied by only .002° C.

From 6 p.m. to 10 p.m. the curve consists of a nearly straight horizontal line. This was part of the time of the electrical preparation. The current had been started in the internal resistance early in the day and the temperature of the outflow water had already become steady at 6 p.m. It is seen that when the evolution of heat is steady, the galvanometer deflection is steady. There is a slight dip in the curve between 9 and 10 p.m. This is accounted for by the observer having been negligent in allowing the current to fall slightly below 1.3 amps.

When L. F. N. entered the calorimeter at 10 p.m. the current was stopped. The small peak in the curve at 10 p.m. is due to the opening of the door which allowed a little warm air to enter. The effect of this had passed off in about 20 minutes.

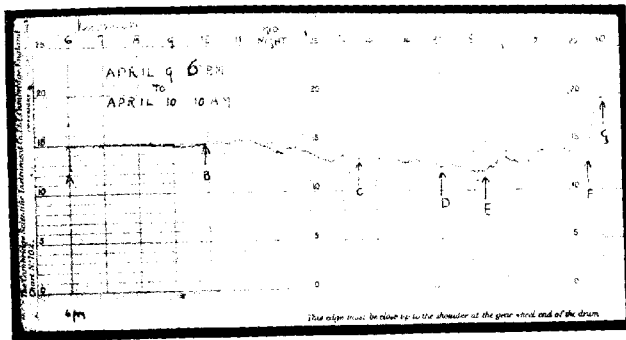


Fig. 3

From 10.30 p.m. to 2.30 a.m. L. F. N. was asleep. The curve during this part consists of two short rises each followed by a longer steady downward slope. L. F. N. is aware that he has the habit of turning over in his sleep, and it is surmised that the rises were due to his having turned over at about 10.45 p.m. and 12.15 a.m. At 2.30 a.m. he awakened and remained awake until about 5 a.m. During this period he made a conscious effort to keep quiet—with fair success as is seen by the curve. At 5 a.m. he went to sleep again and the small irregularities disappeared.

At 6.30 a.m. he awakened and sat up to eat a breakfast of tea and sausages, which he had taken in with him the previous night. The effort of sitting up and eating the food caused a sharp rise in the curve followed by a fall when he lay down. At 7.45 a.m. there was a rapid rise, probably due to the beginning of the specific dynamic action of the food. At

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9.30 a.m. he began exercising as much as was possible in the restricted space, with the result that there was a rapid and great rise in the curve. At 10 a.m. he left the calorimeter.

In the light of later experiments it appears that L. F. N. is an unusually good subject. Most of the subjects who have been in the calorimeter showed much greater deviations from a steady curve.

It should be noted that the irregularities do not give a quantitative picture of the variations in the heat evolution. The total area of a hump in the curve corrected for humidity, etc. must give a correct measure of the total heat that caused it, but the hump is reduced in height and spread horizontally by the high heat capacity of the apparatus. The humps would no doubt be less marked if it were not for the gilled tubing suspended from the roof which absorbs heat more promptly than the walls of the calorimeter.

The next test consisted of the determination of the basal metabolism of eight students of the School of Agriculture.

The subject took his usual breakfast at 8 a.m. and tea and a little bread at about 1 p.m. and 5 p.m., but no other food until the experiment was completed. He entered the calorimeter at 10 p.m. and lay down on a camp bed.

The temperature of the inside of the calorimeter was not quite the same in all the experiments. The lowest was  $15^{\circ}$  C. and the highest was  $17^{\circ}$  C.

The circumstances were not favourable for accurate results as the temperature of the room was in some cases as much as  $5^{\circ}$  below that of the calorimeter, whereby much of the heat was extracted in the ventilating air and in leakage, at the expense of the more accurately measurable heat in the circulating water.

It will be seen by Table IV that the heat extracted in the circulating water was relatively small. As however the results obtained for the basal metabolism are consistent with those obtained by Benedict, Lusk, Cathcart and Orr and others the experiments may be taken as evidence that, even though the corrections are unnecessarily large, they do not involve any considerable loss of accuracy.

Whilst the subject was in the calorimeter hourly readings were taken of the following :

1. Temperature of the air in the room.
2.     "           "     inlet water.
3.     "           "     wet and dry bulbs at air inlet.
4.     "           "     wet and dry bulbs at air outlet.

5. Water flow.

6. Barometer.

7. Manometer showing the difference of pressure between the two sides of the diaphragm by which the air flow was measured.

When an experiment runs satisfactorily these are all practically constant except No. 4. The water flow and the barometer, in fact, need only be read at the beginning and end of the experiment. The humidity

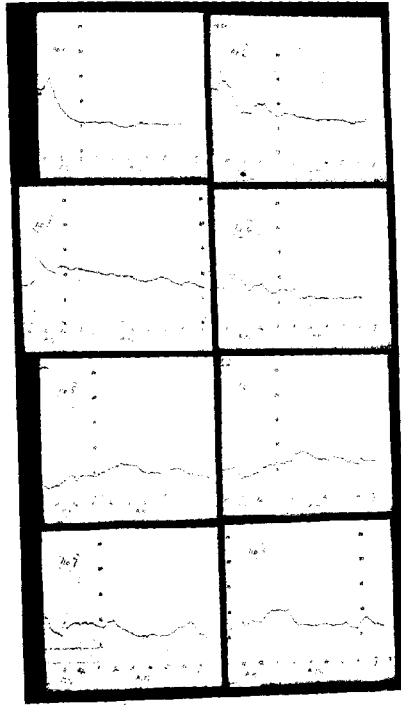


Fig. 4.

of the outlet air rises somewhat rapidly in the first two hours of the test, reaches a maximum after three or four hours, and then slowly falls as the work of digestion falls off and the subject approaches his basal metabolism.

Some later tests with a sheep have shown that if the humidity is read more frequently, fluctuations are seen corresponding to the fluctuations in the temperature of the outflow water. An attempt is being made to get a continuous record of the humidity in order to investigate this.

Fig. 4 is a photographic reproduction of the curves obtained with the eight subjects.

It will be seen that though the subjects made every effort to keep quiet yet the curves obtained are very irregular. In most there are in the latter half fairly straight, horizontal pieces, which doubtless indicate that the subject was sleeping. This horizontal straight line is not necessarily always the lowest part of the curve. If, when the subject has reached his basal metabolism, he pulls the bed clothes over his shoulders or head there is a temporary drop in the evolution of heat. This was actually observed in the case of No. 1. As he had recently gone through an operation and was also suffering from shell shock, the light was turned on momentarily from time to time in order to see whether he was resting quietly. Shortly before 2 a.m. he was seen to pull the sleeping bag completely over his head and this was followed by the dip in the curve between 2.0 a.m. and 3.0 a.m. Such dips, however, are generally slight.

It is clear that, if the basal metabolism is calculated from observations which are made at moderately wide intervals and the mean taken, the result will generally be too high. The chief merit of a continuous record is that it shows the increased metabolism due to movements, and enables us to form an opinion as to which part of the curve most nearly corresponds to the basal metabolism. In the table below showing the results of the experiments, Nos. 3 and 7 are omitted. No. 3 is a peculiar case. The subject had a very trying time during the war and is still suffering from its effects. He remained in the calorimeter for 12 hours, and when let out he had taken no food except tea and a very little bread and butter for 24 hours, yet the curve was still falling. It would have been interesting to keep him in some hours longer, but the state of his health made this somewhat risky. All the subjects except 1 and 3 were in good health. No. 7 is omitted, partly because the curve is very irregular, but mainly because the night was so extremely cold that the temperature of the inlet water could not be kept constant. The town water feeding the supply tank fell so rapidly in temperature that the thermostat went out of action during the night, and had to be readjusted.

Table IV gives the results of the six remaining experiments.

The mean is 37.3—if it is permissible to take the mean of such a small number of results which are well known to vary from one indi-

vidual to another, and probably from day to day with the same individual.

TABLE IV.

	Age	Height	Weight	<i>a</i> Water	<i>b</i> Latent heat	<i>c</i> Leakage	<i>d</i> Air	<i>e</i> Total	Per sq. metre
No. 1	24	5-4½	8-8	-465	-182	-152	-147	-946	35-6
No. 2	24	5-7	9-9	-591	-251	-133	-126	-1-101	38-6
No. 4	21	5-5	8-10	-093	-282	-321	-267	-963	35-8
No. 5	24	6-1	12-5	-209	-408	-376	-293	-1-286	38-2
No. 6	22	5-7	10-4	-112	-401	-248	-325	-1-086	35-7
No. 8	24	5-6½	9-4	-181	-341	-320	-272	-1-124	40-1

Columns *a*, *b*, *c* and *d*, give in kgr. cal. per minute the various quantities of heat which make together the total in column *e*. The last column gives the calories per hour per square metre of body surface, the area being calculated by the Du Bois formula.

Harris and Benedict have given in the Carnegie Institute Publication No. 279 an extensive table of measurements of basal metabolism. If all the males between the ages of 21 and 25 are picked out from this table the mean value is 39-1 with a range from 34-7 to 47-1.

Thus the results obtained are all within the range found by Harris and Benedict for men of similar age.

#### *Defects of the Calorimeter and General Considerations.*

It is clear that in its present state the calorimeter will in favourable circumstances give fairly accurate results. Yet it is not to be regarded as other than an experimental piece of apparatus. Experience has suggested alterations, some of which are so fundamental that they would involve entire rebuilding of the calorimeter. Hence it was thought better to publish a description of the apparatus as it stands, without waiting for a reconstruction which may or may not be made.

Meanwhile it is desirable to note such defects as have revealed themselves up to the present.

Much the most serious defect is the high heat capacity, which makes the calorimeter sluggish in its records, and precludes any except long period experiments.

The actual water equivalent is about 85 kilogrammes.

This has been determined in two ways which give nearly the same result. One method is to calculate the capacity from the weight of the materials and of the circulating water both of which are known approximately.

The second and better method is by a direct determination. Imagine

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the inlet and outlet water to have the same temperature, and a measured current to be started in the resistance wire inside the calorimeter.

The curve traced by the galvanometer needle on the revolving drum is somewhat similar to the curved line  $OQP$ , where  $OY$  represents on the scale of 92.25 mm. per  $1^{\circ}\text{C}$ . the final rise of temperature of the water.

If the calorimeter had no heat capacity, the curve would consist of the two straight lines  $OY$  and  $YP$ . The area  $OYPQO$  represents the heat taken up by the calorimeter when its temperature rises by  $OY$ . The factor for converting square centimetres to calories is easily determined. Draw two ordinates one hour apart cutting the lines  $OX$  and  $YP$ . The area enclosed by these two ordinates and the lines  $OX$  and

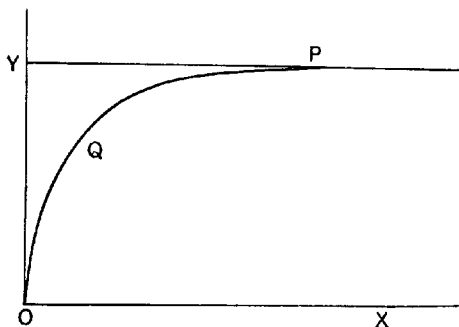


Fig. 5.

$YP$  corresponds to  $F\theta$  where  $F$  is the water flow in one hour and  $\theta$  is the temperature represented by  $OY$ . Then the area of the figure  $OYPQO$ , measured by the planimeter or otherwise, gives the heat required to raise the calorimeter by  $\theta^{\circ}\text{C}$ .

This high capacity has the effect of smoothing out more or less any humps on the curve due to fluctuations in the heat evolution of an animal in the calorimeter—an effect which must be present to some extent in any calorimeter.

The radiator tube was fixed in series with the helical circulating tube and suspended near the roof of the calorimeter in order to give at least a qualitative indication of fluctuations. It appears to answer this purpose, for since it was added any slight fluctuations in the heat evolution show themselves on the curve more plainly than they did before.

The heat capacity could be materially reduced by using a smaller

tube for the circulating water. The tube would weigh less and it would contain less water. The tube in use at present is  $\frac{3}{4}$  in. bore. A tube of  $\frac{1}{4}$  in. bore would be ample, and would have the further advantage of reducing the risk of air-locks. The water flow is limited by the consideration that if it is too great the rise of temperature is small, and the accuracy of measurement of the differences of temperature is diminished. In general about 2 litres per min. is found suitable. This gives a low velocity in a  $\frac{3}{4}$  in. pipe, but in a  $\frac{1}{4}$  in. pipe it would be high enough to drive out accumulations of air.

There would be some further advantage if the pipe were *not* wound round the tank in vertical planes as at present, but in a nearly horizontal direction, gradually rising from the bottom to the top, for there would then be a single point at which all the air would gather, and this air could be released through an air cock when necessary; or a vertical tube of sufficient height to prevent outflow of water could be fixed at the highest point to get rid of air as it gathered, as was done in some earlier calorimeters constructed by Professor A. V. Hill.

If some such arrangement were adopted it would not be necessary to place the supply tank so high above the calorimeter. This would get rid of the long supply pipe, which gives trouble in very cold weather by allowing the water to cool on its way down. It would be a decided improvement if the calorimeter room were lofty enough to permit of the supply tank being in it.

A thinner gauge of metal might with advantage be used. A rectangular tank of sheet copper  $\frac{1}{16}$  in. in thickness, stayed where necessary, and provided with a wooden floor would be strong enough for the purpose and would have a smaller heat capacity.

It should be noted that the heat capacity of a calorimeter constructed as is the present instrument is not strictly a constant, but depends on the rate of change of the temperature. Part of the heat is taken up by the cork insulation, which is slow in reaching a steady state. If the fluctuations are rapid the heat penetrates only a little way into the cork and the virtual capacity is less than it is when the changes are so slow that the cork is always approximately in an equilibrium state.

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## THE EVAPORATION OF WATER FROM SOIL. II. INFLUENCE OF SOIL TYPE AND MANURIAL TREATMENT.

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(With Two Text-figures.)

### INTRODUCTION.

IN an earlier paper<sup>1</sup> a study was made of the evaporation of water both from sands and soils, using an apparatus devised to overcome sources of error in the work of previous investigators. The results showed that the essential difference in the evaporation from sand and soil was primarily due to the colloidal proportion of the clay fraction in the soil, and secondly, to the organic material present. A portion of a rich garden soil from which the soluble humus had been extracted in the usual way with 2 % NaOH after previous acidification, was remoistened; evaporation took place at only a slightly greater rate than from the unextracted soil. But evaporation from another portion of the soil, remoistened after ignition to a dull red heat to destroy the colloidal property of the clay and to remove all organic matter, was practically indistinguishable from that given by moist sand. Further support for this view was given, on the one hand, by the similar behaviour of ignited and unignited silt fractions, which suggested that the insoluble organic matter was not a controlling factor, and on the other hand, by the similar character of the evaporation curves of ignited soil and a china clay which possessed only feeble colloidal properties.

Differences in evaporation of the same nature as mentioned above should be shown by soils containing different amounts of clay and by soils which have received a number of different manurial treatments.

The present paper describes experiments—using the same apparatus and technique as before—done on two soils, one of which contains about 6 % of clay, and the other 15 %. In order that the influence of manurial treatments could be studied at the same time, samples were taken on each soil from the following plots: the unmanured; farmyard manure; complete artificial manure.

<sup>1</sup> B. A. Keen, *J. Agric. Sci.* 6 (1914), p. 456.

## DESCRIPTION OF SOILS USED.

The soils were taken from the permanent barley plots of Hoos Field, Rothamsted and the permanent wheat plots of Stackyard Field, Woburn. A number of borings were made in the 0-9" layer on each of the six plots, using the 2" cylinder. The soil for each plot was thoroughly mixed, so as to give a representative sample for the experiment. The mechanical analyses of the six soils are given in Table I.

Table I. *Rothamsted, Hoos Field (Permanent Barley Plots), 0-9".*

	Unmanured	Complete minerals and ammonium salts	Farmyard manure
Fine gravel ... ..	1.65	1.09	1.78
Coarse sand ... ..	10.86	9.50	7.86
Fine sand ... ..	24.35	28.02	25.30
Silt ... ..	22.38	22.89	20.96
Fine silt, I ... ..	7.95	8.90	8.29
Fine silt, II ... ..	1.93	2.11	2.68
Clay ... ..	15.22	15.35	15.71
Ignition loss ... ..	8.41	7.19	11.72
Solution loss ... ..	6.85	3.54	5.67
Totals ... ..	99.60	98.59	99.97

*Woburn, Stackyard Field (Permanent Wheat Plot), 0-9".*

	Unmanured	Complete minerals and ammonium salts	Farmyard manure
Fine gravel ... ..	—	—	—
Coarse sand ... ..	51.95	62.63	50.77
Fine sand ... ..	22.34	13.12	22.30
Silt ... ..	7.35	5.36	7.51
Fine silt, I ... ..	3.95	5.87	3.38
Fine silt, II ... ..	0.79	0.91	0.97
Clay ... ..	5.89	6.52	8.82
Ignition loss ... ..	4.82	4.72	5.62
Solution loss ... ..	1.77	2.74	3.32
Totals ... ..	98.86	101.87	100.69

The Hoos Field soils contained sufficient water when sampled for the experiments to be done on the soil<sup>1</sup> in its natural moist condition. As the Woburn soils contained less than 10 % of water when sampled, this was increased to about 20 % by the addition of distilled water from a bulb sprayer.

## EXPERIMENTAL RESULTS.

A considerable number of experiments was done on each of the six soils and the mean curve obtained for each in the manner described in the earlier paper. The values from which these mean curves are constructed are given in Table II.

<sup>1</sup> All the soils used in these experiments were passed through a 3 mm. sieve beforehand.

Table II.

Percentage of water	Rothamsted, Hoos Field			Woburn, Stackyard			
	Unmanured	Complete minerals and amm. salts	Farmyard manure	Unmanured	Complete minerals and amm. salts	Farmyard manure	
		Time in hours and decimal fractions of hours					
15	0.00	.00	.00	.00	.00	.00	
14	.12	.11	.13	—	—	—	
13	.23	.22	.27	.190	.182	.176	
12	.26	.34	.41	—	—	—	
11	.50	.47	.55	.366	.363	.364	
10	.64	.59	.71	.486	.456	.456	
9	.79	.73	.87	.589	.553	.566	
8	.95	.87	1.05	.691	.656	.676	
7	1.12	1.04	1.25	.810	.762	.790	
6	1.30	1.19	1.46	9.33	.876	.920	
5	1.51	1.36	1.69	1.06	.993	1.05	
4	1.75	1.56	1.96	1.23	1.13	1.20	
3	2.03	1.79	2.30	1.42	1.30	1.36	
2	2.37	2.10	2.76	1.61	1.51	1.56	
1.6	2.60	2.25	3.03	—	—	—	
1.5	—	—	—	1.77	1.64	1.72	
1.2	2.89	2.50	3.49	—	—	—	
1.0	3.11	2.66	3.86	1.98	1.84	1.96	
.8	3.43	2.91	4.40	2.14	1.98	2.14	
.6	3.94	3.34	5.37	2.47	2.20	2.51	
.4	4.83	4.30	6.83	3.45	2.99	3.71	

Inspection of the figures in Table II shows, firstly, that the three Hoos Field soils evaporate more slowly than the Woburn soils. The latter are, in fact, intermediate between the sand and Hoos Field curves given in the previous paper. The difference between the Woburn and Hoos Field soils is in reality greater than shown in Table II, where the initial percentage of moisture is for convenience taken in all cases as 15 %. Actually, the Hoos Field soil is relatively drier than the sandy Woburn soil when each contains 15 % of water. As nearly as could be judged from the appearance of the soil and the shape of the rate of evaporation curves, which will be referred to later, the soils were equally moist when the Hoos Field soil contained about 30 % water and the Woburn soil 12 % and the fundamental comparison between the two soils should be made on this basis. But although the actual differences are not so large, the comparison is made more convenient by adjusting the results to the same initial moisture content. This becomes more apparent when the data given in Table II are plotted in Fig. 1. The figure also brings out clearly a second point of interest in Table II—the effect of manurial treatment on the evaporation. Taking the three Hoos Field soils, the farmyard manure soil is slowest, the artificial manure soil is fastest and the unmanured soil, which has not been plotted

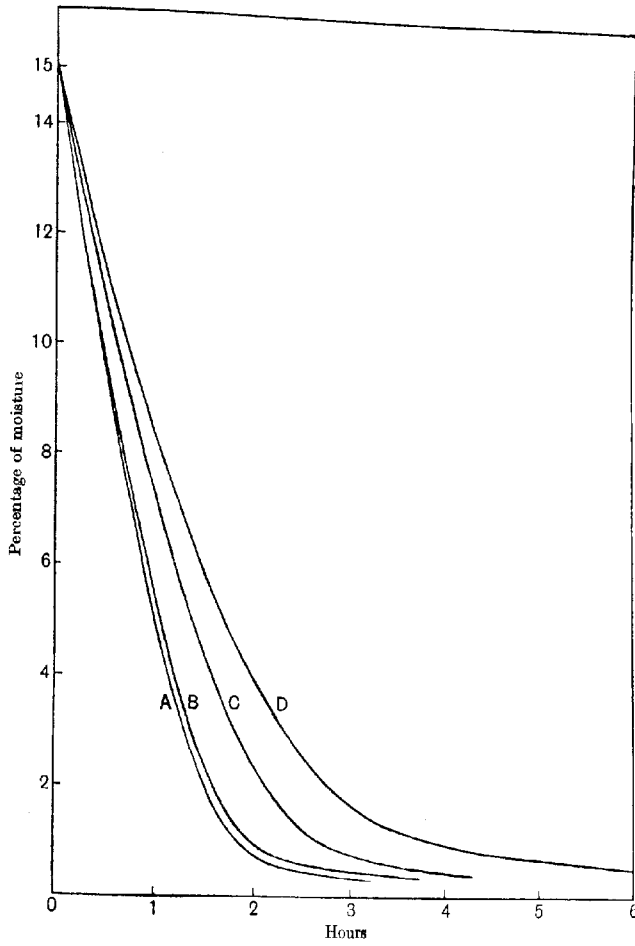


Fig. 1. Evaporation curves for: Woburn soil, complete minerals and ammonium salts (A).  
 " " farmyard manure (B).  
 Rothamsted soil, complete minerals and ammonium salts (C).  
 " " farmyard manure (D).

in order to avoid confusing the figure, occupies an intermediate position. The effect of manuring on the evaporation from Woburn soil is quite small. There is an indication that, as in the Hoos Field soils, the plot

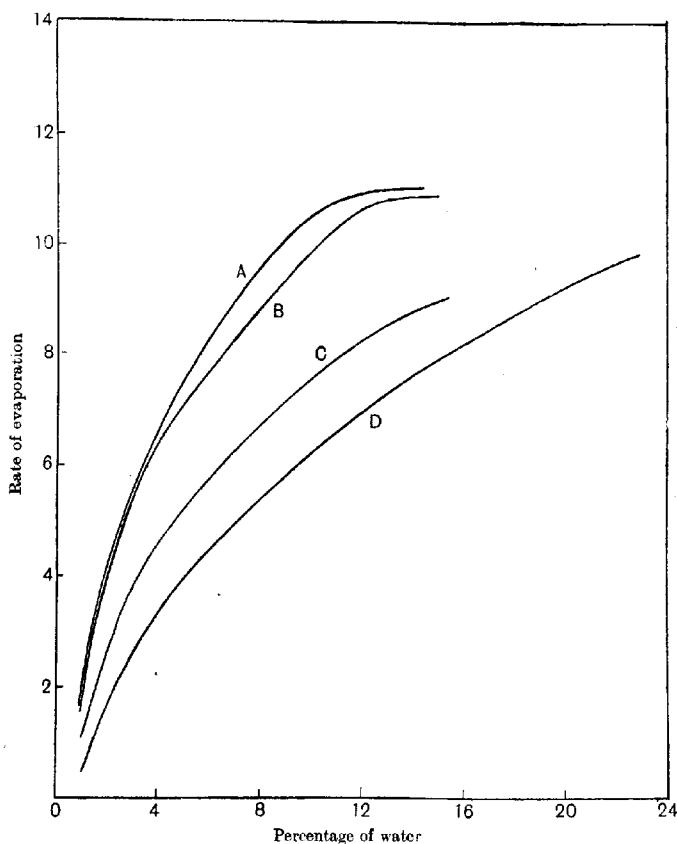


Fig. 2. Evaporation curves for: Woburn soil, complete minerals and ammonium salts (A).  
 „ „ „ farmyard manure (B).  
 Rothamsted soil, complete minerals and ammonium salts (C).  
 „ „ „ farmyard manure (D).

receiving artificial manure gives the fastest evaporation, but the unmanured and farmyard manure plots are identical within experimental error.

Although it was improbable that the evaporation from Woburn soils would be in any way affected by the addition of water mentioned above, the point was tested by air-drying some of the soil, after which it was re-moistened, and evaporation experiments done on it in the usual way. No differences in evaporation could be detected between the soil so treated and that used in its natural condition. A few preliminary experiments were also made on the effect of previous air-drying on the evaporation from the Hoos Field soil, and no striking differences were observed.

These general differences between the various soils are shown up clearly by the rate curves (Fig. 2) where the time rate of evaporation is plotted against the percentage of water. The curves lie in two groups determined by the type of soil and within these groups there are differences associated with the manurial treatment. All the Woburn soils at low percentage of water are indistinguishable from one another: over the range 4-12 % the artificial manure plot has the highest rate of evaporation and at higher percentages the rates for the three soils are nearly the same. The small differences in the rate above 12 % are of course the consequence of the small differences between the corresponding figures in Table II. At percentages of water in excess of approximately 12 % the rate of evaporation remains constant. The inference is that above 12 % evaporation is taking place as it would for a free water surface. Below 12 % there are slight differences attributable to the form of manuring, but on a sandy soil these differences are very slight. This is not the case in the Rothamsted soils containing more clay. Although the curve is primarily determined by the soil type, yet the effect of manuring is also evident, the three curves being distinct over their whole course.

It may be concluded that the effect of manuring on the evaporation rate is more pronounced the more clay there is in the soil. It has already been shown that the surface of the particles from which the moisture evaporation takes place possesses a colloidal structure, which is largely determined by the clay fraction of the soil. The greater the amount of clay the more opportunity is there for modifications of this surface by the action of the different manures.

The curves show a gradual decrease in rate of evaporation as the percentage of moisture diminishes. There was not sufficient moisture in the artificial manure and unmanured plots when sampled to give the initial flat portion of the rate curve, but the farmyard manure plot contained very nearly enough.

Comparison of the experimental or rate curves with the mechanical analyses of the soils (Table I) is interesting. In the three Hoos Field soils the clay fraction is practically constant. From other evidence it is possible that the fraction fine silt II, whose upper limit of diameter is .005 mm., has some of the properties of the clay fraction. It is, in fact, included with clay in the American system of mechanical analysis.

The sum of clay and fine silt II for the Hoosfield soils is:

Unmanured	Artificial manure	Farmyard manure
17.15	17.46	18.39

These differences are small, and not in the same order as the evaporation curves, in which the unmanured, not the artificial manured, soil is intermediate. On the other hand, the ignition losses, which in view of the similar clay contents in the three plots can be taken as roughly proportional to the content of organic matter, are in the same order as the evaporation curves.

The sum of the clay and fine silt II fractions of the Woburn soil are:

Unmanured	Artificial manure	Farmyard manure
6.68	7.43	9.79

which are in the same order as the clay fractions alone. The order of the unmanured and artificial manure plots is reversed for the ignition losses, which, however, do not differ much among themselves.

On comparison of these figures with the experimental curves there are no very definite relations. This is probably due to the comparatively small amounts of clay and organic matter present in these soils, and the preponderance of the larger sized particles. In this connection it should be noticed that although the sum of the two fractions coarse sand and fine sand is nearly the same for each of the three soils, the artificial manure plot has considerably more of the coarser fraction than the other two plots, and this may explain the fact why it evaporates quicker than both the farmyard manure and the unmanured plots. When the rate curves were being discussed reference was made to the linear portion, parallel to the axis of moisture content, which indicated that over this range evaporation was taking place as it would from a free water surface. This linear portion eventually gives place to a characteristic portion over which the rate of evaporation progressively decreases in moisture content. The approximate transition point between these sections of the curve is of interest because it occurs at the percentage of moisture at which the influence of the soil particles on the evaporation enters directly. Among the number of soil constants which have been devised mainly

by American investigators it was thought that the moisture equivalent<sup>1</sup> would possibly be of interest in this connection.

Through the kindness of Mr G. R. MacDole, now of the Iowa Experiment Station, U.S.A., the moisture equivalents of some of the soils used in these experiments have been determined:

Hoos Field			Woburn		
Unmanured	... ..	0-9", 21.2	Unmanured	... ..	10-6", 12.1
Artificial manure	... ..	0-9", 20.4		... ..	7-12", 10.8
Farmyard manure	... ..	0-9", 25.8	Farmyard manure	... ..	10-6", 13.5
				... ..	7-12", 12.9

Comparison of these figures with the curves of Fig. 2 brings out the fact that the moisture equivalent for the Woburn soils corresponds fairly closely with the point at which the rate of evaporation first begins to decrease.

The moisture equivalents for the Hoos Field soils are in the same order as the corresponding curves in Figs. 1 and 2, *i.e.* the soil with the lowest moisture equivalent evaporates most quickly. Unfortunately none of the Hoos Field soils contained sufficient water to show up the initial straight portion of the rate curves, hence a direct comparison of the moisture equivalent and the flat portion of the rate curve cannot be made. But it is fairly obvious from the rate curve and the values in Table I that a moisture percentage of 24 % in the farmyard manure soil is only slightly below that at which the flat portion of the rate curve would appear.

The available evidence, therefore, suggests that the moisture equivalent of a soil measures the approximate percentage of moisture at which the soil particles directly influence the evaporation of water. This possibility is implied in the statement of Briggs and McLane that the centrifugal force used in the determination removes the water from the larger interstices of the soil and leaves only that in intimate contact with the soil particles. In this connection however it should be noticed that the moisture equivalent will vary with the thickness of the soil layer used, owing to the difference in centrifugal force from top to bottom of the layers. The experimental details worked out by Briggs and McLane were used by McDole for the soils considered here, so that the comparison which has been made above is with the moisture equivalent as determined by the standard method.

<sup>1</sup> Briggs and McLane, U.S. Bureau of Soils, *Bull.* 45 (1907).



## SUMMARY.

Further experiments have been done on the evaporation of water from soil, using the same apparatus and technique as described in an earlier paper. The present series of experiments was designed to investigate the effect of clay content and manurial treatment on the evaporation. Two soils have been used, one containing only 6 % clay and the other 15 %, and from each soil samples were taken from plots which had received (a) no manure, (b) artificial manure, (c) farmyard manure. The rate at which the soils lost water over concentrated sulphuric acid and at a constant temperature, was found to depend firstly on the amount of clay present, and secondly on the amount of organic material in the soil. The differences due to content of organic material were more obvious in the soil containing the larger amount of clay; the farmyard manure plot lost water at the slowest rate, and the unmanured plot occupied an intermediate position. In the sandy soil the differences in evaporation due to manuring were small.

There is evidence that the moisture equivalent of these soils measures the percentage of water at which the evaporation is first directly affected by the soil particles, and that at percentages of water in excess of the moisture equivalent evaporation is taking place substantially from a free water surface.

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# THE RELATION BETWEEN THE CLAY CONTENT AND CERTAIN PHYSICAL PROPERTIES OF A SOIL.

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(With Five Text-figures.)

## INTRODUCTION.

It is well known that the behaviour of most soils is largely determined by the percentage of clay they contain. A considerable amount of work has been done from this point of view, some of which has been already discussed by one of us<sup>1</sup> and hence need not be repeated here.

In the present paper a simple method is described for measuring various fundamental properties of soil, such as pore space, apparent and real specific gravity, volume expansion and so on. The results for successive depths of one soil are given here for illustrative purposes. They have proved of sufficient promise to warrant the application of the method to a large variety of soils, and we have been fortunate in enlisting the aid of the Science Masters Association who have arranged for tests to be made at a number of schools.

## DESCRIPTION OF SOIL USED.

The soil was obtained from Mr Alfred Amos, of Spring Grove, Wye, Kent, from a deeply cultivated portion of a hop garden. It was taken some years ago, and was kept tightly corked in an air-dry condition until used. Six successive depths of the soil were separately stored as follows: 0-6", 6-12", 12-18", 18-24", 2-3', 3-4'. The mechanical analysis of each depth is shown in Table I. For reasons which are mentioned below, each sample was passed through a sieve of 100 meshes to the linear inch before use and the figures refer to the soil passing the sieve.

<sup>1</sup> B. A. Keen, *J. Agric. Sci.* **10** (1920), 44.

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Table I. *Mechanical analysis of successive depths of the same soil.*

Depth	...	...	0-6"	6-12"	12-18"	18-24"	2-3'	3-4'
<i>Fraction:</i>								
Fine sand	...	...	28.2	33.8	51.7	41.0	45.0	54.6
Silt	...	...	37.3	37.2	21.3	28.7	24.6	21.4
Fine silt, I	...	...	9.5	6.7	7.5	4.7	5.2	1.7
Fine silt, II	...	...	2.8	1.8	.3	1.6	2.2	.8
Clay	...	...	10.2	10.1	16.7	17.1	15.3	14.1
Solution loss	...	...	3.95	3.9	2.95	2.6	2.2	2.9
Loss on ignition	...	...	7.5	6.7	6.0	4.55	3.8	4.0
Totals	...	...	99.45	100.2	99.70	100.25	98.3	99.5

### EXPERIMENTAL METHOD.

A number of small brass boxes are used, made as follows: a piece of stout brass sheet  $8" \times 1"$  is bent into four sides of a box, 2" square by 1" deep, and the join carefully soldered. Another portion of the metal is made into a detachable bottom piece slightly larger than 2" square and provided with a turned up edge about  $\frac{1}{2}"$  high which should fit closely round the first portion. The bottom is pierced with 11 rows of 11 holes each about .75 mm. in diameter and .5 cm. apart. A square of filter paper which is cut to the internal measurement of the box-bottom is placed therein, and held firmly by the lower edge of the sides of the box when this is placed in position. The box is weighed and is then filled with the sieved and air-dry soil in a systematic manner, so that the method of packing may be as uniform as possible. From 8-10 grms. are added at a time and the box tapped on the bench after each addition. When the box is nearly full, sufficient soil is added to allow the surface to be struck off flat with a spatula. The upper edges of the box are then in turn tapped smartly with the edge of the spatula, and more soil is added. This is struck off flat as before and the process repeated until very slight settling of the soil occurs, when the surface is finally struck off flat and the box and contents weighed. The box and contents are then placed in a flat bottomed dish containing about  $\frac{1}{4}"$  depth of distilled water and left over night. When a number of boxes are placed in the same dish, additions of water must be made at intervals to keep the level constant. The behaviour of the soil while moistening is taking place is interesting. Considerable movement takes place<sup>1</sup>, the top of the block of soil retreating from the sides of the box before it becomes moist, and at the same time rising in height. Eventually when the interstices are saturated the wet soil expands back to the sides of

<sup>1</sup> In some cases this initial movement is accompanied by a cracking of the surface soil. These cracks may persist when the soil is saturated. It is not yet certain whether they are solely due to slight variations in the method of packing; some soils show the effect more than others.

the box but the vertical expansion remains. The next morning the boxes are rapidly dried on the outside, weighed, and replaced in the water for a few minutes. The portion of the soil which has expanded above the top of the box is then removed as follows: an ordinary razor blade held at a slight angle with the horizontal is placed along one edge of the box and then drawn across the top. The removal of the soil is facilitated if, previous to the use of the razor, a flat-edged spatula is used to divide the expanded soil into three rectangular blocks, of approximately equal volume. Each block is then removed in turn with the razor. This surplus soil is transferred from the razor to a small glass or metal dish as cleanly as possible, and weighed. Any soil adhering to the under side of the razor should not be put into the dish but replaced on soil in the brass box, to which it belongs. The box and residual soil are weighed after the outside has been dried, and then placed, together with the dish containing the surplus soil, in a water oven for 24 hours<sup>1</sup>. At the end of this period they are cooled in a desiccator and weighed again.

In addition to these weighings it is necessary to measure carefully the internal volume of each brass box, which is done by taking a series of measurements of the height, breadth and length, and averaging; also to determine in the usual way the moisture present in the air-dry soil used in the experiments; to measure the amount of water taken up by the wet filter paper which is best done by taking six squares, saturating them with water, removing the surplus with a glass rod, and measuring the increase of weight due to the water remaining.

It will be found convenient to have a rectangular metal box and lid in which the brass box and soil—whether air-dry or saturated—are placed for weighing purposes.

The necessary weighings and measurements are therefore:

Weight of weighing box, brass box, and filter paper	...	...	<i>a</i> grms.
" " " " air-dry soil	...	<i>b</i>	"
" " " " wet " saturated soil	...	<i>c</i>	"
" " " " " (saturated)	...	<i>d</i>	"
" " " " " (residual)	...	<i>e</i>	"
" " " " " (oven-dry)	...	<i>f</i>	"
" " " " " (residual)	...	<i>g</i>	"
" metal dish (or watch glass)	...	<i>h</i>	"
" " " " " saturated surplus soil	...	<i>i</i>	"
" " " " " oven dry	...	<i>j</i>	"
" " " " " " "	...	<i>k</i>	"
Percentage of moisture present in air-dry soil	...	...	p.c.c.
Internal volume of brass box	...	...	...

<sup>1</sup> In view of the large mass of soil to be dried, heating for 48 hours at 100° C. was also tried; 24 hours was found to be long enough.

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From these weighings the following results are obtained:

$$(1) \text{ Weight of unit volume (100 c.c.) of air-dry soil (apparent specific gravity) } = \frac{b-a}{c} \times 100.$$

$$(2) \text{ Amount of water taken up by unit weight of soil } = \frac{(c-a) - (b-a)}{(b-a)}.$$

*Note.* Correct  $(b-a)$  for the  $x\%$  of water contained in the air-dry condition, and  $(c-a)$  for the water in the filter paper.

$$(3) \text{ Pore space } = \frac{\text{Volume of water in box}}{\text{Volume of box}} = \frac{(d-a) - (c-a)}{r}.$$

*Note.* Correct  $(d-a)$  for the water in the filter paper.

$$(4) \text{ Specific gravity of soil } = \frac{(e-a)}{v - (d-p)}.$$

$$(5) \text{ Volume expansion of 100 c.c. of soil } = \frac{\text{Volume of water in saturated surplus soil} + \text{volume of surplus soil}}{\text{Volume of box}}$$

$$= \frac{(g-h) + \frac{h-f}{\text{sp. gravity}}}{r} \times 100.$$

As mentioned above, the soil on which these measurements are made is that passing the sieve having 100 meshes to the inch. A number of trials were made with the soil passing the 1 mm. sieve, but concordant results could not be obtained. This is due not so much to the larger soil particles but to the varying amounts of soil crumbs or compound particles passing the sieve. The presence of these particles undoubtedly affects the packing of the soil into the boxes, and consequently all the subsequent weighings. The results discussed below apply therefore to a soil whose mechanical analysis differs somewhat from that of the unsieved soil in having a greater percentage of the finer particles. For the majority of ordinary soils the difference is not serious, but it is appreciable on soils containing a larger percentage of fine sand. It is probable that in this case the better experimental procedure would be to sieve the soil through the 100 mesh sieve so as to break up any compound particles, and then to remix this portion with that which passed the 1 mm. sieve but was held by the 100 mesh. It is intended to experiment in this direction not only on sandy soils but on those containing more clay.

#### DISCUSSION OF RESULTS.

The experimental figures of duplicate determinations for the successive depths of soil are given in Table II. Columns (5) and (6) have been corrected for the water taken up by the filter paper. A measure of the experimental error in slicing off the surplus soil with the razor, is given by comparing the values in column (5) with the sum of the corresponding figures in columns (6) and (7). There should be no difference; actually there is a small loss in each case varying from .01 gm. to .63 gm. The

average loss is under .25 gm. and well within experimental error from other sources. After a little practice, the maximum loss can be kept well below .25 gm. From the values in Table II the various constants

Table II.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Depth of soil	Vol. of brass box in cu.cms. (v)	Wt. of air-dry soil in grms. (b-a)	% moisture in air-dry soil x	Wt. of satd. soil in grms. (c-a)	Wt. of satd. soil less surplus in grms. (d'-a)	Wt. of satd. surplus soil in grms. (g-f)	Wt. of oven-dry soil less surplus in grms. (e-a)	Wt. of oven-dry surplus in grms. (h-f)
0-6"	68.7 66.7	79.93 79.16	3.2	116.55 114.86	111.60 109.50	4.82 5.24	75.09 74.13	2.99 3.23
6-12"	67.9 68.2	79.26 79.39	2.2	115.44 115.49	111.46 111.91	3.88 3.57	75.14 75.49	2.41 2.20
12-18"	67.1 67.9	78.86 79.34	3.2	120.96 121.38	105.89 105.38	14.44 15.62	68.01 67.60	8.97 9.70
18-24"	68.4 67.4	79.39 76.62	3.15	121.75 118.63	106.80 104.69	14.72 13.55	68.05 66.15	9.08 8.24
2-3'	67.8 68.3	80.16 81.98	2.85	120.60 122.75	107.66 109.94	12.65 12.57	70.21 72.14	7.99 7.90
3-4'	68.1 68.1	85.21 87.82	3.3	124.67 127.12	112.06 113.77	12.37 13.13	75.66 77.47	8.94 8.58

Table III.

Constants		Depths					
I		0-6"	6-12"	12-18"	18-24"	2-3'	3-4'
Wt. of unit vol. (100 c.c.) of air-dry soil. (Apparent specific gravity)	Duplicates	116.3 118.7	116.7 116.4	117.5 116.8	116.0 113.7	118.2 120.0	125.0 129.0
	Average...	117.5	116.5	117.1	114.8	119.1	127.0
II							
Amount of water taken up by unit weight of soil	Duplicates	.50 .50	.49 .49	.58 .58	.58 .60	.55 .54	.51 .49
	Average...	.50	.49	.58	.59	.545	.50
III							
Pore space	Duplicates	53.1 53.0	53.5 53.4	56.4 55.5	56.7 57.2	55.3 55.3	53.5 53.5
	Average...	53.05	53.45	55.95	56.95	55.3	53.5
IV							
Specific gravity	Duplicates	2.33 2.36	2.38 2.38	2.33 2.24	2.29 2.29	2.31 2.36	2.39 2.44
	Average...	2.345	2.38	2.285	2.29	2.335	2.415
V							
Volume expansion of unit volume of soil	Duplicates	4.53 5.07	3.65 3.36	13.9 15.1	14.0 13.2	11.05 11.75	11.3 11.85
	Average...	4.80	3.50	14.5	13.6	11.85	11.57

indicated above are easily determined. They are given in Table III and are plotted against the corresponding percentage of clay in Figs. 1-5. In each of these figures the actual duplicates are shown as crosses and

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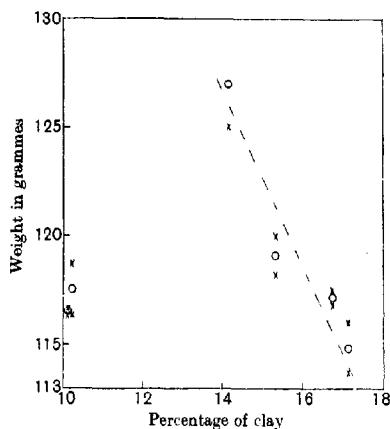


Fig. 1. Relation between clay content and weight of unit volume of air-dry soil.

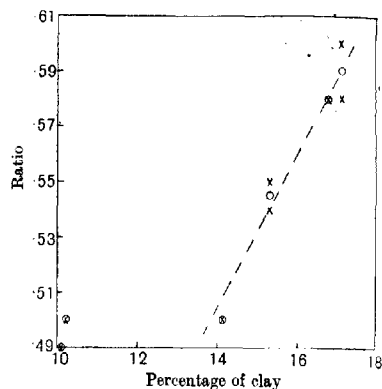


Fig. 2. Relation between clay content and ratio:  $\frac{\text{weight of water taken up}}{\text{weight of soil}}$ .

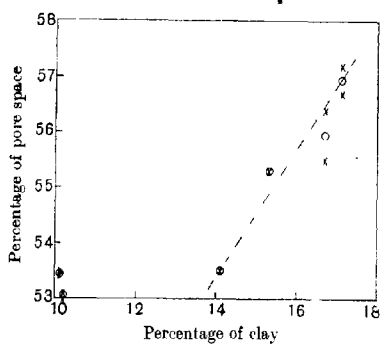


Fig. 3. Relation between clay content and percentage of pore space.

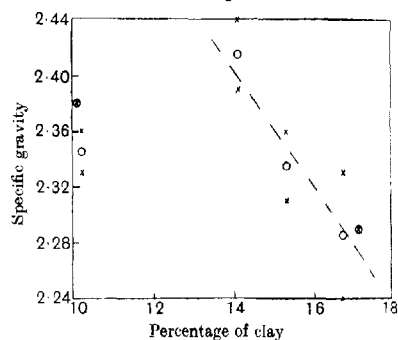


Fig. 4. Relation between clay content and specific gravity of soil.

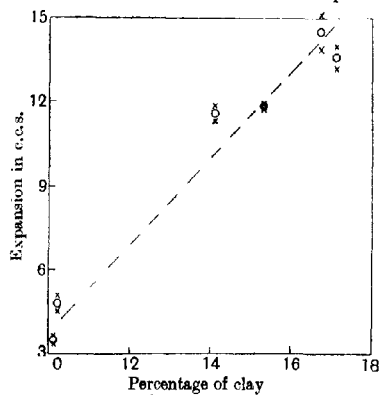


Fig. 5. Relation between clay content and volume expansion of soil

mean of each pair as a circle. In interpreting the relations of the experimental results to the percentage of clay it must be remembered that the soil contains considerably more organic matter in the top 12" than in the lower depths; hence the top layer will in some respects behave as if its clay content were in excess of the value given in Table I. Ignoring for the moment the values for the 0-6" and 0-12" layers and paying attention to the remaining four depths, it will be seen that in each of the Figs. 1-5 there exists a general relationship between the amount of clay and the variations in the given constant. The straight line in each figure is, of course, only an indication of the general trend of the values. The apparent and real specific gravities (Figs. 1 and 4 respectively) show an inverse relationship with the percentage of clay, while the amount of water taken up by the soil (Fig. 2), the pore space (Fig. 3), and, to a lesser extent, the volume expansion (Fig. 5) are directly related to the clay percentage. The effect of the organic matter is shown in Figs. 1-5 by the soils corresponding to the 0-6" and 6-12" layers, which contain just over 10 % of clay. These two layers give values for the various constants very similar to those of the layers containing more clay except in the case of the volume expansion (Fig. 5). Elsewhere it will be shown that, if the bottom layer of this soil be assumed devoid of organic matter, the approximate percentages in the top two layers are 3.7 and 3.95 respectively. Inspection of Figs. 1-5 in detail shows that the apparent and real specific gravities (Figs. 1 and 4) of the top two layers are equivalent to the values given by the layer with 15 % of clay, while the pore space (Fig. 3) and amount of water taken up by the unit weight of soil (Fig. 2) correspond to 14 % of clay. The volume expansion (Fig. 5) is apparently not affected by the organic matter. The organic matter therefore is, weight for weight, equivalent to clay except in the volume expansion measurements, where its possible effect is within the experimental error.

The fraction fine silt II (upper limit of diameter .005 mm.) possesses similar properties to the clay, and if included with it, does not appreciably affect the order of the above results. In most soils this fraction is not present in considerable quantity.

There are a number of other points brought out by the further inspection of the tables and diagrams.

In the calculation for the ratio: weight of water taken up by a given weight of soil, the weight of the whole of the soil in the box was used. It is possible to obtain similar ratios for both the surplus soil (that which was removed by the razor) and the residual soil (that



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remaining in the box). These ratios are obtained from Table II in the forms

$$\frac{\text{column 7} - \text{column 9}}{\text{column 9}} \quad \text{and} \quad \frac{\text{column 6} - \text{column 8}}{\text{column 8}},$$

respectively.

It will be found that the values for the residual soil are slightly below those for the total soil given in Table III, while those for the surplus soil are considerably in excess. There are a number of possible causes which may account for this. In addition to the water taken up by the surplus soil there is an obvious film of water on the surface of the soil which will increase the above ratio for the surplus soil; the lower layers of soil, being confined by the rigid brass box, can only swell vertically against the resistance of the superincumbent layers so that the ratio for the residual soil will be diminished; there is probably some air trapped in the interstices of the soil as the water ascends. It is not likely that the presence of air is the sole cause of difference, because the amount of air so trapped would be very variable from box to box, whereas the experimental results show very little erratic variation. It is possible by suitably manipulating the figures to obtain an idea of the amount of trapped air, as follows:

The calculation of the absolute specific gravity of the soil was obtained from the expression:

$$\frac{\text{wt. of residual soil}}{\text{vol. of box} - \text{vol. of water}}$$

The low values of the specific gravity (Table III) can be explained by the presence of air, which reduces the volume of water. Taking the true value of the specific gravity to be 2.70, it is easy to calculate for any given soil what should be the volume of water completely filling the pore space. The difference between this volume and the experimental figure may be regarded as the volume occupied by the trapped air. Taking for illustration the first duplicate of the 0-6" depth (*i.e.* the top row of figures in Table II) we obtain

$$2.70 = \frac{75.09}{68.7 - x},$$

$$x = 40.9 \text{ c.c. of water.}$$

The experimental figure is  $111.60 - 75.09 = 36.51$  c.c.; *i.e.* 4.4 c.c. are occupied by air, which is equivalent to 6.4 % on the total volume of the brass box. Turning now to the already mentioned variation in the ratio of weight of water taken up to weight of soil we find from

Table II that in the surplus soil it is  $\frac{1.83}{2.99} = .61$  and in the residual soil  $\frac{36.51}{75.09} = .49$ .

Assuming that the value for the residual soil has been reduced solely owing to the air entrapped, and that the true value is .61, 75.09 grms. of soil should take up 45.8 grms. of water, instead of 36.51, the experimental value. Using this value of 45.8 grms. of water for  $x$  in the calculation of the specific gravity immediately above, we obtain the figure 3.28. This is obviously incorrect. Hence the diminished value in the residual soil of the ratio of weight of water taken up to weight of soil, cannot be wholly due to the entrapping of air within the box. The other possible causes have been already mentioned above.

#### SUMMARY.

A simple experimental method has been described for measuring certain physical constants of soil, using small brass boxes into which soil passing a sieve of 100 meshes to the inch has been packed by hand. The quantities determined are:

- (1) The weight of unit volume (100 c.c.s.) of air-dry soil, or the apparent specific gravity.
- (2) Amount of water taken up by unit weight of soil.
- (3) Pore space.
- (4) Specific gravity of the soil.
- (5) The volume expansion of unit volume (100 c.c.) of soil when saturated.

The results for one soil only are given, and discussed, to illustrate the method. With the co-operation of the Science Masters Association it is being applied to a number of soils by various schools.

The particular soil used was obtained in six depths as follows: 0-6", 6-12", 12-18", 18-24", 2-3', 3-4', and the above constants were determined on each depth. It was shown that (1) and (4) varied inversely with the percentage of clay in the soil, while (2), (3), and (5) varied directly with the clay percentage. The effect on the constants of the larger quantities of organic matter present in the top two layers of soil was, weight for weight, approximately equal to that of the clay, except in the volume expansion results where the effect if any was within experimental error.

It is possible that the fraction fines silt II, whose upper limit of diameter is .005 mm., has similar effects to the clay fraction.

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## THE FLOCCULATION OF SOILS. II.

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IN a previous paper<sup>1</sup> on the flocculation of soils attention was drawn to the fact that calcium salts flocculate the clay of soils very quickly in an alkaline medium and relatively slowly in a neutral medium. In so far as the flocculation of suspended particles is a consequence of the neutralization of their electric charge by oppositely charged ions or colloids, and as alkalinity is known to increase the negative charge on suspended particles, the flocculation of clay by calcium hydroxide is manifestly abnormal. The suggestion was put forward in the earlier paper that the clay particles are heavily "protected" by members of the so-called emulsoid group of colloids, and that their behaviour is accordingly thrown out of line with that of a relatively simple system of suspended particles. Such a conception of the hydrophilous surface of the clay particles is in accordance with the prevalent view that has arisen in other ways<sup>2</sup>. A clay particle suspended in water is believed to be a system in which there is a gradual transition from the solid nucleus through stages of gel material to the water. What has been called the "protection" of the clay particle is not to be regarded as the mere coating of the particle: the emulsoid matter is intimately and indissolubly related to the particle: it is a part of the particle, merging into the less hydrated matter on the one hand and into water on the other.

Now if transitional stages of emulsoid matter protect the particle and relate it to the water in the manner indicated, then theoretically there are at least three possible types of action whereby a clay suspension may become flocculated when an electrolyte is introduced into the surrounding water, namely,

1. *Direct or normal flocculation* due to the action on the particle of an ion or colloid of opposite sign: that is, an action qualitatively the same as would occur if there were no protection, and which is merely retarded by the protective colloid.

<sup>1</sup> Comber, *Journ. Agric. Sci.* **10** (4), 1920.

<sup>2</sup> See Keen, *Journ. Agric. Sci.* **10** (1), 1920.

2. *Indirect flocculation* which will take place if the added substance enters into a reaction with constituents of the particles giving rise to products which then act as flocculants.

3. *Abnormal flocculation*, such as would occur if the flocculant had a coagulating or precipitating action on the protective colloid or material absorbed thereby.

In the present communication it is proposed to consider the flocculation of soil clay by various reagents in regard to these three headings. In so doing it is not, of course, intended to suggest that the action of any one reagent is solely confined to one of the above named types.

#### EXPERIMENTAL.

##### A. Flocculation by iron and aluminium compounds.

1. A London Clay subsoil was treated with 5 per cent. HCl, washed and dried. A suspension of the clay fraction was then obtained by the process used in the mechanical analysis of soil (but without the use of ammonia). The suspension used in these experiments contained 0.68 gm. of ignited dry matter per litre.

A series of 12 tubes (cleaned and calibrated as described in the earlier paper) was then arranged, each tube containing 10 c.c. of the suspension. In a parallel series of tubes a colloidal solution of ferric hydroxide was placed in amounts varying from 0 to 12 mgms.  $\text{Fe}_2\text{O}_3$  per tube, and the volume in each tube was made up to 5 c.c. The ferric hydroxide in each tube was then added rapidly to the corresponding tube containing clay suspension. Each of the tubes, containing clay in constant amount and ferric hydroxide in varying amount in a fixed volume of 15 c.c., was inverted three times and the whole series was then allowed to stand. Within five minutes a voluminous precipitate was forming in the tube containing 3 mgms.  $\text{Fe}_2\text{O}_3$ ; the tubes containing more and less showed no evidence of flocculation. The tubes immediately on either side of the one containing 3 mgms.  $\text{Fe}_2\text{O}_3$  showed signs of flocculation after an hour. The suspensions flocculated in sequence with increasing or decreasing concentration of ferric hydroxide. With each repetition of the experiment the point in the series at which flocculation was at an optimum was clearly marked.

This experiment was repeated with a similar and equally pronounced result, using aluminium hydroxide in place of ferric hydroxide.

2. The foregoing experiment was also carried out using solutions of ferric chloride and of aluminium chloride instead of the hydroxides.

A point of optimum flocculation was found when the solution of the chloride was added quickly and the tubes shaken immediately after the addition of the chloride. When the chloride was added slowly and when mixing was delayed concentrations greater than the optimum all caused flocculation like the optimum. The best result was obtained and the optimum effect always shown when the clay suspension was added to the flocculant. The optimum concentration of  $\text{AlCl}_3$  for the London Clay suspension here used was about  $\frac{N}{2000}$ .

3. The relative flocculating power of aluminium salts and the corresponding acid was examined at that concentration at which the flocculating power of the aluminium salt is greatest. Ten c.c. of the clay suspension was put into each of six tubes. To each of three of these 5 c.c. of  $\text{AlCl}_3$  solution was added so that the final concentration of that salt was  $\frac{N}{2000}$ . Five c.c. of  $\text{HCl}$  of the same concentration was added to each of the other three tubes. Flocculation had commenced in the tubes treated with  $\text{AlCl}_3$  after standing 30 minutes and in 90 minutes the coagulum had completely settled. Flocculation did not commence in the acid-treated tubes until after 12 hours. The use of  $\text{Al}_2(\text{SO}_4)_3$  and  $\text{H}_2\text{SO}_4$  gave a similar result. The aluminium salt, when used at the concentration required for its maximum effect, is a very much better flocculant than the corresponding acid.

All the foregoing experiments on the flocculation of clay by iron and aluminium compounds were repeated with an Oxford Clay (the suspension containing 0.78 gm. per litre) and a sample of Fuller's Earth (the suspension containing 0.18 gm. per litre). The results were qualitatively the same, the hydroxides and chlorides of iron and aluminium showing, in the same way, a point of optimum flocculation in their series, and the aluminium salts flocculating far better than the corresponding acid.

#### *B. The effect of $\text{CO}_2$ on the flocculation of clay by $\text{Ca}(\text{OH})_2$ .*

1. Experiments are described in the earlier paper in which two similar clay suspensions are flocculated, one with a neutral calcium salt and the other with  $\text{Ca}(\text{OH})_2$ —the  $\text{Ca}(\text{OH})_2$  giving the better flocculation. This experiment was repeated with a number of different clays, and after flocculation,  $\text{CO}_2$  was passed through the  $\text{Ca}(\text{OH})_2$ -treated tube. The tubes were then gently shaken and their contents again allowed to flocculate. The system treated with the neutral salt was now found to flocculate slightly more quickly than the other, which behaved exactly

as if treated with  $\text{Ca}(\text{HCO}_3)_2$  in the first instance. The superior flocculation of clay by  $\text{Ca}(\text{OH})_2$  is reversible to  $\text{CO}_2$ .

2. This reversibility to  $\text{CO}_2$  suggests the possibility of the flocculation of clay being due to the formation of  $\text{CaCO}_3$ . The following experiments were made in examination of this suggestion.

(a) Twelve tubes were arranged, six containing various clay suspensions (10 c.c. in each tube) and six each containing 10 c.c. distilled water. To the water in the latter six tubes  $\text{CO}_2$  was added in amounts varying from a partial replacement of the superincumbent air to a complete saturation of the water. 1 c.c.  $\frac{N}{25}$   $\text{Ca}(\text{OH})_2$  was added to each of the twelve tubes, which were shaken and allowed to stand. The clay suspensions flocculated as previously described. In four of the  $\text{CO}_2$ -solution tubes there was a turbidity but nothing comparable with the voluminous precipitate in the other tubes.

(b) A number of clay suspensions were flocculated by  $\text{Ca}(\text{OH})_2$ . By means of a dropping pipette one drop of dilute  $\text{HCl}$  was brought into contact with the settled coagulum. The coagulum was dispersed but without effervescence.

(c) A number of clay suspensions were flocculated by  $\text{Ca}(\text{OH})_2$ , and for comparison a similar series was flocculated by  $\text{CaCl}_2$ . The coagula were allowed to settle and to remain undisturbed for one month. No diminution in the volume of the coagula produced by  $\text{Ca}(\text{OH})_2$  could be detected during the month. The tubes were gently inverted at the end of the month and again allowed to stand. The coagula settled out very much as before.

Flocculent precipitates of  $\text{CaCO}_3$  formed by adding  $\text{Ca}(\text{OH})_2$  to solutions of  $\text{Na}_2\text{CO}_3$  were found to lose their flocculent condition and to become fine powders in a very few minutes.

(d) Several clay suspensions were each divided into two 10 c.c. portions. One portion of each was treated with  $\frac{N}{25}$   $\text{Ca}(\text{OH})_2$  and the other portion with  $\frac{N}{25}$   $\text{Ba}(\text{OH})_2$ . So far as could be seen there was no difference between the action of  $\text{Ca}(\text{OH})_2$  and that of  $\text{Ba}(\text{OH})_2$ . It was found however—and the fact is well known—that there is an enormous difference between the action of equivalent amounts of  $\text{Ba}(\text{OH})_2$  and  $\text{Ca}(\text{OH})_2$  on dilute solutions of carbonate or of carbonic acid.  $\text{Ba}(\text{OH})_2$  gives a bulky precipitate when  $\text{Ca}(\text{OH})_2$  gives only a faint turbidity.

C. *The effect of OH ions on the flocculation of clay by Ca ions.*

The following experiments were first carried out with a deep London Clay which was practically devoid of organic matter. The clay suspension was prepared in the usual way and was found to contain 0.87 gm. ignited clay per litre.

1. Two 10 c.c. portions of the clay suspension were taken and one burette drop of  $\frac{N}{4}$   $\text{NH}_4\text{OH}$  was added to one of them. The peptization of the clay by the alkali produced a marked visible effect. The effect was very clearly seen by transmitted light, in which the neutral suspension was relatively cloudy, while the alkaline suspension approached, in appearance, the clarity of a solution. The whole effect of the  $\text{NH}_4\text{OH}$  is not brought about instantaneously. This was shown by adding one drop of  $\frac{N}{4}$   $\text{NH}_4\text{OH}$  to the tube not previously thus treated, after one minute. The tube which received the  $\text{NH}_4\text{OH}$  before the other was distinctly clearer at first but after five or six minutes the appearances of the tubes were alike. The experiment was repeated several times and with varying amounts of  $\text{NH}_4\text{OH}$ . The results indicate quite consistently that  $\text{NH}_4\text{OH}$  peptizes clay in suspension, that the peptization proceeds during a measurable period of time to a maximum and that the action is more rapid the greater the amount of  $\text{NH}_4\text{OH}$  added.

2. To 10 c.c. of the clay suspension were added 1 c.c.  $\frac{N}{25}$   $\text{NH}_4\text{OH}$  and after inverting once 1 c.c.  $\frac{N}{25}$   $\text{CaCl}_2$ . To another 10 c.c. of the suspension were added 1 c.c.  $\frac{N}{25}$   $\text{Ca}(\text{OH})_2$  and 1 c.c. distilled water. The tubes were inverted three times together and allowed to stand. The tube which received the  $\text{NH}_4\text{OH}$  first and the  $\text{CaCl}_2$  afterwards flocculated before the tube which received the Ca ions and the OH ions together. To ensure that the  $\text{NH}_4$  and Cl ions were not responsible for this difference, the experiment was repeated using 2 c.c. of an equal mixture of the  $\frac{N}{25}$   $\text{NH}_4\text{OH}$  and the  $\frac{N}{25}$   $\text{CaCl}_2$  in place of the  $\text{Ca}(\text{OH})_2$ . The result was as before. Further, it is clearly impossible for one manipulator satisfactorily to treat two clay suspensions differently at the same instant: therefore this, and all similar experiments, were carried out repeatedly, alternating the order in which the two treatments were made. All repetitions showed that adding the OH ion before the Ca ion caused a more rapid and more voluminous flocculation than adding the two together. Adding the two together, however, caused a more rapid and more voluminous flocculation than adding a neutral calcium salt alone.

3. By a procedure similar to that of the preceding experiment, the effect of adding the OH ion first and then the Ca ion was compared with the effect of adding the Ca ion first and then the OH ion. The addition of the OH ion first invariably gave the better flocculation of clay. There appeared to be little difference between the effect of adding the Ca ion first and then the OH ion, and that of adding the two together.

4. An experiment was described in the earlier paper in which the effect of varying the amount of  $\text{NH}_4\text{OH}$  upon the subsequent flocculation of clay by a neutral calcium salt was examined. The range of the final concentrations of  $\text{NH}_4\text{OH}$  in that experiment was  $\frac{N}{30}$  to  $\frac{N}{3}$  and variations over that range did not appear to influence the flocculation. Further experiments have now been made with lower concentrations of  $\text{NH}_4\text{OH}$ . A series of tubes each containing 10 c.c. of the clay suspension was arranged and  $\text{NH}_4\text{OH}$  added in concentrations varying from  $\frac{N}{2000}$  to  $\frac{N}{80}$ . Up to concentrations of about  $\frac{N}{200}$ , increase in the concentration of the  $\text{NH}_4\text{OH}$  caused an enhancement of the flocculation by 1 c.c.  $\frac{N}{25}$   $\text{CaCl}_2$ . Beyond a concentration of about  $\frac{N}{200}$   $\text{NH}_4\text{OH}$  there was no apparent difference in the flocculation.

5. The influence of the time of contact of the  $\text{NH}_4\text{OH}$  and the clay upon the subsequent action of  $\text{CaCl}_2$  was examined. To one of two similar suspensions of the clay,  $\text{NH}_4\text{OH}$  was added in such amount as to bring the final concentration to  $\frac{N}{250}$ . The tube was shaken and allowed to stand for five minutes. The same amount of  $\text{NH}_4\text{OH}$  was then added to the other suspension which was shaken. One c.c.  $\frac{N}{25}$   $\text{CaCl}_2$  was then added to each suspension. After shaking, the tubes were allowed to stand. Flocculation was almost immediate in the tube which received  $\text{NH}_4\text{OH}$  first, and many repetitions of the experiment consistently showed that with small amounts of  $\text{NH}_4\text{OH}$ , the time of contact—within certain limits—affected the subsequent action of  $\text{CaCl}_2$  on the clay.

6. It was shown in the earlier paper that when a sample of soil is shaken with sufficient water, a suspension is formed, the flocculation of which by calcium salts is retarded by the addition of alkali: but if the suspension be allowed to stand for a period of time which varies from soil to soil, the material still remaining in suspension can be flocculated by calcium salts better if alkaline. Now a number of experiments have been carried out in which 10 gm. of various soils have been stirred with a column of water 8.5 cm. high, and the portion decanted after various



periods of time has been examined to find the turning point after which clay dominates the system so that it flocculates better with alkaline than neutral calcium compounds. In no instance was any sharply defined turning point found: but it was found that for each soil there is a range of mixtures of clay and larger particles which show a superior flocculation by calcium salts in alkaline suspension during the first stages of flocculation and an inferior flocculation afterwards. Thus, the suspension decanted from 10 gm. of the deep London Clay at any time between 10 and 15 minutes from the beginning of sedimentation was examined by adding 1 c.c.  $\text{CaCl}_2$  to 10 c.c. of the suspension in one tube and 1 c.c.  $\text{CaCl}_2$  with one drop  $\frac{N}{4} \text{NH}_4\text{OH}$  to 10 c.c. of the suspension in another tube. The alkaline suspension began to flocculate before the neutral one, but it afterwards lagged behind the neutral one, the flocculation of which began last and finished first.

7. The critical mixtures of clay and larger particles described in the preceding paragraph were examined as described in paragraphs 2 and 3 of this section to find the effect of adding the OH ion and the Ca ion at different times. It was found in every such experiment that the addition of the Ca ions first and then the OH ions produced a much more rapid flocculation of the system than the addition of the Ca ions after the OH ions. This is the reverse of the behaviour of clay and is quite different from the behaviour of silt<sup>1</sup>.

*D. The effect of the previous treatment of a clay suspension with alkali upon its subsequent flocculation with acid.*

1. The experiments described in paragraphs 2 and 3 of section C immediately preceding, were carried out with substitution of 1 c.c.  $\frac{N}{10} \text{HCl}$  instead of the  $\text{CaCl}_2$ . It was found that previous treatment of the clay suspension with 1 c.c.  $\frac{N}{25} \text{NH}_4\text{OH}$  very greatly enhanced the flocculating power of 1 c.c.  $\frac{N}{10} \text{HCl}$  added afterwards. The addition of the  $\text{NH}_4\text{OH}$  after the acid and the addition of an equivalent mixture of  $\text{NH}_4\text{Cl}$  and  $\text{HCl}$  produced a flocculation not visibly different from that due to the  $\text{HCl}$  alone.

The whole of the experiments described in sections C and D were repeated with an Oxford Clay containing appreciable amounts of humus.

<sup>1</sup> The whole of the experiments recorded in section C, as well as some others, have since been repeated with other soils by Mr G. Walsh, student in the University of Leeds, who is investigating some of the possible practical applications of these studies. Mr Walsh's results entirely confirm those recorded here.

The results throughout were qualitatively the same as with the deep London Clay.

*E. The effect of pulverization upon the flocculation of silt.*

1. A small quantity of the unignited fine silt fraction of a Garforth (Coal Measures) soil was subjected to a wet-grinding in an agate mortar for about one hour. Suspensions of the product were then made and the effect of alkalinity upon the flocculation of such suspensions by calcium salts was then examined by the method previously described. The suspension behaved like clay, being much more readily flocculated from alkaline than neutral suspension.

The experiment was repeated with the Palaeozoic Silt Loam soil referred to in the earlier paper. The pulverization of this soil was not carried sufficiently far to enable suspensions to show the clay behaviour fully. After two hours grinding, suspensions behaved like the "critical mixtures" of clay and silt previously described.

*F. The flocculation of silica suspensions.*

1. A suspension of Kahlbaum silicic anhydride was prepared as in the experiments described in the earlier paper. With this suspension practically all the experiments previously described for clay were carried out. It is unnecessary to record here the experimental detail, which was quite similar to that of the clay experiments. The salient results are:

(a) Silica is not flocculated from suspension, nor is the suspension visibly affected, by neutral calcium salts, by aluminium or iron salts, by acids, or by  $\text{NH}_4\text{OH}$ .

(b) Silica is flocculated by  $\text{Ca}(\text{OH})_2$ , and the flocculation is reversed by  $\text{CO}_2$ . It is also flocculated by a calcium salt in conjunction with an alkali, but there is no apparent difference between the effect of adding either first or both together.

(c) The subsequent flocculation by a calcium salt is not apparently affected by the concentration of the  $\text{NH}_4\text{OH}$  previously added to the suspension, nor by the length of time the  $\text{NH}_4\text{OH}$  alone remains in contact with the silica.

(d) Silica is flocculated from suspension by acid after previous treatment with alkali.

(e) The behaviour of silica as above described is unaffected by previous ignition at a red heat.

2. A silicic acid sol containing after dialysis 25 gm.  $\text{SiO}_2$  per litre, was precipitated by  $\text{Ca}(\text{OH})_2$ . Carbon dioxide was then passed in, but

there was no evidence of a re-solution of the gel. This experiment was performed with increasing dilutions of the sol but so long as a visible precipitate was formed with  $\text{Ca}(\text{OH})_2$  there was no apparent removal of the precipitate by  $\text{CO}_2$ .

3. One hundred c.c. of the silicic acid sol was evaporated on a water bath and dried in a steam oven to the consistency of glass. The residue was ground in a mortar, and suspensions were prepared. These suspensions were flocculated by  $\text{Ca}(\text{OH})_2$  and the flocculation was found to be reversible to  $\text{CO}_2$ .

4. Ten gm. ignited kaolin were shaken with 100 c.c. dilute silicic acid sol (containing 2.5 gm.  $\text{SiO}_2$  per litre) and the whole was evaporated on a bath and subsequently dried in the oven. At various times during the period of drying portions were removed and suspensions made. The suspensions were flocculated by  $\text{Ca}(\text{OH})_2$  and the effect of  $\text{CO}_2$  examined. While the consistency of the material was liquid or pasty, the  $\text{Ca}(\text{OH})_2$  flocculation was not reversed by  $\text{CO}_2$ , but after further drying the material formed suspensions the flocculation of which, by  $\text{Ca}(\text{OH})_2$ , was reversible to  $\text{CO}_2$ .

#### G. *The action of $\text{Ca}(\text{OH})_2$ in low concentrations on clay.*

1. A series of 24 tubes was arranged each containing 10 c.c. of the London Clay suspension. Eleven of these tubes received  $\text{Ca}(\text{OH})_2$  in such amounts that the final concentration varied from  $\frac{N}{5000}$  to  $\frac{N}{450}$ . Two tubes were untreated, and the remaining eleven were treated with  $\text{CaCl}_2$  in the same concentrations as the  $\text{Ca}(\text{OH})_2$ . In concentrations greater than  $\frac{N}{1400}$  the  $\text{Ca}(\text{OH})_2$  flocculated better than the  $\text{CaCl}_2$  as previously noted and discussed, but in concentrations below  $\frac{N}{1400}$  the  $\text{CaCl}_2$  was by far the better flocculant, indeed the  $\text{Ca}(\text{OH})_2$  treated suspensions appeared to be deflocculated as by treatment with  $\text{NH}_4\text{OH}$ .

### DISCUSSION.

#### *The Normal Flocculation of Soil Clay.*

*Flocculation by iron and aluminium compounds.* Experiments described above show that when a number of similar clay suspensions are treated with colloidal aluminium hydroxide, or ferric hydroxide, in amounts which increase from one end of the series to the other, it is easy to detect a point in the series at which flocculation is most rapid. On either side of this point flocculation is less marked. With reasonable

care the similar occurrence of a point of maximum flocculation can be demonstrated when the chloride of iron, or of aluminium is used. These results are analogous to those obtained by Burton<sup>1</sup> in his experiments on the coagulation of platinum and silver sols by aluminium salts. Beyond the point of maximum flocculation the metallic hydroxide protects the particles and produces a stable positively charged system.

The diminution of flocculation which occurs after a certain concentration of iron or aluminium compound is passed can only be seen when the flocculant is added quickly. If it is added drop by drop, that is if incipient flocculation is permitted, there is no reversal of the flocculation.

Hence it appears that the flocculation of clay by aluminium and iron compounds falls into line with the isoelectric point theory.

#### *The Indirect Flocculation of Soil Clay.*

*Flocculation by neutral salts.* When solutions of neutral salts are brought into contact with soils an exchange of bases takes place between the soil and the solution. Potassium salts, for instance, are generally very efficient in this respect being absorbed from solution in relatively large amounts while calcium, magnesium, etc.,—accompanied by aluminium if the soil is sour—are given up to the solution. This fact of base exchange must have some disturbing action on the course of a normal flocculation due to the potassium ion. Some part of the flocculation brought about by the addition of a potassium salt to a clay suspension will be really due to calcium, aluminium, etc., brought into solution during the exchange of bases.

Such indirect flocculation is not easily amenable to experimental demonstration, but in view of the known facts of base exchange it seems inevitable that flocculation of clay by a neutral salt is complicated accordingly. The quantitative interpretation of flocculation experiments which involve the use of reactive substances such as clay and even kaolin<sup>2</sup>—the experiments of Bödlander<sup>3</sup> and of Hall and Morison<sup>4</sup> for instance—cannot therefore be regarded as simple.

With calcium salts in low concentrations such complication may not be great, but it is likely to be much more pronounced with, for example, potassium salts.

*Flocculation by acids.* Again, it seems probable that the power of

<sup>1</sup> Quoted in Burton, *The Physical Properties of Colloidal Solutions*, 1916.

<sup>2</sup> Regarding the reactivity of kaolin see Sullivan, *U.S. Geol. Sur. Bull.* No. 312, 1907.

<sup>3</sup> *Jahrb. Mineral.* 1893, 2.

<sup>4</sup> Hall and Morison, *Journ. Agric. Sci.* 2, 1907.

acids to flocculate clay is largely indirect. Dilute acids bring alumina and other bases into solution from soils, and these bases may then be a cause of flocculation.

It is commonly held that acids flocculate better than any salts, and this may be urged against the view that dissolved bases are an important ulterior cause of flocculation by acids. Now in view of the fact that there is an optimum concentration of aluminium salt for the flocculation of a given clay suspension and that concentrations greater than this optimum protect and stabilize the suspension, there is little obvious meaning in the relative flocculating powers of aluminium salts and acids when the concentration of the flocculants is chosen at random. Experiments have been described above in which the comparison has been made at concentrations in the neighbourhood of that required for optimum flocculation by aluminium salts. The experiments show very decidedly that under those conditions of concentration the aluminium salts are very much better flocculants. In consideration of that consistent result the suggestion is here made that the flocculating power of acids is largely due to the action of aluminium, etc., which the acids bring into solution. This, of course, is exactly opposite to the more frequent suggestion which assumes some mechanism for flocculation by acids and attributes flocculation by aluminium salts to the acid produced by the hydrolysis of these salts.

Hall and Morison (*loc. cit.*) obtained some interesting results of experiments on the flocculation of suspensions of bauxite and some similar minerals. They found that sulphuric acid and magnesium sulphate flocculated while aluminium sulphate in equivalent concentration stabilized the suspensions. This anomaly may be due to the protective action of the aluminium salt.

#### *The Abnormal Flocculation of Soil Clay.*

Under this heading it is proposed to consider flocculation by calcium hydroxide and by calcium salts in conjunction with alkali, continuing the considerations of the earlier paper. The crucial fact that the presence of hydroxyl ions enhances the flocculation of clay by calcium salts was described in that earlier paper, and in quite general terms the explanation was suggested that this anomaly is due to reactions between the calcium salts and the emulsoid surface of the clay particles: for it is known that alkalinity expedites reactions between calcium salts and such hydrophilous colloids as silicic acid. It is here intended to give more detailed consideration to this suggested explanation.

*The reversibility of the calcium hydroxide flocculation of clay  
to carbon dioxide.*

Before further consideration is given to the theory already advanced, it is desirable to give attention to the possibility of carbonate formation as a cause of the flocculation of clay by calcium hydroxide. Hall and Morison (*loc. cit.*) allude to the complication which arises in experiments on flocculation by calcium hydroxide on account of the formation of carbonate which entrains the particles. The complete exclusion of carbon dioxide is certainly difficult and after all possible precautions have been taken there still remains the possibility of an unknown amount of carbon dioxide being absorbed in the soil particle<sup>1</sup>. Moreover, experiments have been described above which show that the flocculation of clay by calcium hydroxide is reversible to carbon dioxide and this lends colour to the possibility of carbonate formation being a cause of the anomalous flocculation in question.

Several experiments have been made in critical examination of this point. The experiments establish the following facts which show with reasonable certainty that carbonate formation does not play a significant part in the experiments in which clay is flocculated by calcium hydroxide.

1. When calcium hydroxide is added to water containing carbon dioxide there is no precipitate which can be imagined to cause, by entrainment, the marked and voluminous flocculation which occurs when the same amount of calcium hydroxide is added to a clay suspension.

2. When dilute hydrochloric acid is added to the precipitate formed by adding calcium hydroxide to a clay suspension there is no effervescence such as would be expected if carbonate had been formed in sufficient amount to entrain the particles.

3. The bulky precipitate resulting from the action of calcium hydroxide on a clay suspension will remain without any apparent alteration for a long time—certainly for several weeks. Moreover if the tube is gently inverted after several weeks standing the precipitate settles out again very much as it did originally and without any marked diminution of volume. Now it is well known that when calcium carbonate is first precipitated it is very bulky but that after a few minutes it loses its bulkiness and becomes a fine powder.

4. In equivalent concentrations the hydroxides of calcium and barium have, as far as can be observed, the same effect on clay suspensions but their visible effects on water containing carbon dioxide or carbonate are enormously different.

<sup>1</sup> See Russell and Appleyard, *Journ. Agric. Sci.* 7 (1), 1915.

5. Previous treatment of the clay suspension with ammonium hydroxide facilitates flocculation by acids as well as by calcium compounds, and carbonate formation is clearly out of the question when excess of acid is present.

6. Suspensions of silica can be flocculated by calcium hydroxide and the action reversed by carbon dioxide. The previous ignition of the silica, which may be expected to remove absorbed gases, has no apparent effect on the action of calcium hydroxide.

These facts seem to make it clear that carbonate formation cannot contribute more than a minor action to the flocculation of clay by alkaline calcium compounds.

Further consideration may now be given to this action of carbon dioxide, for the purpose of giving more detail and precision to the theory already advanced in explanation of the flocculation of clay by lime. In attributing that flocculation to a reaction with emulsoid or hydrophilous colloids the writer has in the earlier paper and elsewhere<sup>1</sup> argued mainly from the analogous action of alkaline calcium compounds on colloidal silica. Silica has been named as a type of the emulsoid colloids concerned and also as one likely to be present in considerable amounts. The suggestion is that the precipitation of clay by calcium hydroxide is akin to the precipitation of silica by calcium hydroxide. Now the precipitation of a *silica sol* by calcium hydroxide is apparently irreversible to carbon dioxide. Calcium bicarbonate will not precipitate the dilute sol but once it has been precipitated by calcium hydroxide, treatment with carbonic acid does not appear to cause re-dissolution, whereas the flocculation of clay by calcium hydroxide is easily reversed by carbonic acid. Further, when fine powders such as ferric oxide or kaolin are suspended in a silica sol there is a rapid and voluminous flocculation following the addition of calcium hydroxide but there is no reversal of the flocculation when carbonic acid gas is added. On the other hand it was found that when suspensions of Kahlbaum silicic anhydride, of dried and powdered silica gel, or of kaolin evaporated with silica sol and dried in the oven are flocculated by calcium hydroxide the flocculation is readily reversed by carbonic acid. There are two extremes of a silica-water system, namely, a clear sol which forms with calcium hydroxide a precipitate which does not appear to be re-dissolved by adding carbonic acid, and a suspension of silica gel which forms with calcium hydroxide a coagulum which is fairly readily reversed by carbonic acid. The transition from one extreme to the other can be observed by the addition of dilute silica sol to a

<sup>1</sup> Comber, *Trans. Faraday Soc.* **17**, 1921.

suspension of an impalpable powder (*e.g.* ignited kaolin) and the examination of the system at various stages of drying. By the experiments described it is shown that the flocculation produced by calcium hydroxide is apparently irreversible to carbonic acid up to a certain stage in the drying process and that it then becomes reversible. It is very doubtful whether there is anything essentially different between the action of calcium hydroxide on silica sol and its action on silica gel. Le Chatelier<sup>1</sup>, in his examination of the precipitate formed by adding calcium hydroxide to dilute silica sols, found that when that voluminous precipitate is washed with water, all the calcium can be removed leaving a pure silica gel, but he also remarks that not all the silica so remains, some having been removed in the washing. Therefore and because it is generally recognized that no hard and fast line can be drawn between reversible and irreversible coagulations, it has been said above that the precipitation of a silica sol by calcium hydroxide is *apparently* irreversible.

There arises from this consideration the first point which it is desired to make in elaboration of the theory that the flocculation of clay by calcium hydroxide is largely due to the action of the hydroxide on silica and similar emulsoids, namely that it is not due to the action of the hydroxide on a silica sol present in gross amount, but to the action of the hydroxide on the surface of an emulsoid gel. A system of particles coated by a silica gel more nearly represents soil clay than does a suspension of particles in a silica sol, but the action of calcium hydroxide on either of these systems is not fundamentally different from its action on the other since it is an essential part of the conception of a hydrophilous colloid that its interface with the dispersion medium is an incipient sol. This consideration seems to bear directly upon the interesting problem of the absorption of bases by "solid" acids, which however cannot be pursued here.

It may be worth while to consider the foregoing conception of clay flocculation from another point of view. It has been enunciated by Fucks<sup>2</sup> that when, in a suspension of particles in a liquid, the attraction of the particles for the liquid exceeds that of the particles for one another, the suspension will persist because the nett result will be a mutual repulsion of the particles, whereas if the attraction of the particles for the liquid is less than that of the particles for one another the particles will be flocculated because the nett result will be a mutual attraction of the particles. Now a strong mutual attraction between a substance

<sup>1</sup> Le Chatelier, *La Silice et les silicates*, 1914.

<sup>2</sup> Cited from Burton, *The Physical Properties of Colloidal Solutions*, 1916.



and water is an essential part of the definition of an emulsoid or hydrophilous colloid and the stability of suspensions of silica and of clay will be due—or partly due—to the attraction of the particles for the dispersion medium. Since a calcium salt in an alkaline medium precipitates silica from its solutions it will presumably do so in the zone of attraction which characterizes the interface silica-water (and clay-water). That is to say it will remove the condition resulting from the silica-water (and clay-water) attraction and by thus reducing the attraction of the particles for the liquid will permit the flocculation of the particles in accordance with Fucks' laws.

*The effect of hydroxyl ions upon the action of calcium ions on clay.*

In addition to, and in further search for the explanation of, the main fact of the earlier paper (namely that hydroxyl ions facilitate the flocculation of clay by calcium ions) a number of facts are established by the experiments described above.

In the first place it is shown that clay differs from silica by being:

- (a) flocculated by acids, aluminium salts and neutral calcium salts,
- (b) visibly deflocculated by alkalis,

and that clay resembles silica by being:

- (i) flocculated by calcium salts more easily in alkaline medium (silica is not flocculated by calcium salts otherwise),
- (ii) flocculated by acids more readily after the previous addition of ammonium hydroxide (silica is not flocculated by acids otherwise).

Now these points of difference and resemblance (as well as the other experimental facts) seem to be easily explicable on the assumption that clay is composed of particles whose surface is made up of such emulsoids as silica. Stated crudely, the siliceous coating explains the points of resemblance to silica and the "core" of the particle explains the points of difference.

The chief point of difference, namely the flocculation of clay by acids, aluminium salts and neutral calcium salts, is thought to take place, as previously discussed, by direct or indirect action between the flocculant and the particles. The surface colloids may play some secondary part such as retarding the action between the flocculant and the clay, or even receiving the absorbed part of the flocculant and forming the same absorption compounds as when the flocculants react primarily with them under the influence of alkali. But the main action seems to concern the particles and not their colloidal surfaces. Silica alone is not flocculated by acids, aluminium salts and neutral salts; but then it is, so to speak,

all emulsoid matter with no electrically charged and chemically reactive "core" beneath it as there is in clay.

The relation between the surface material and the core of the particle will be more fully discussed further on. In this section it is submitted that there are two primary mechanisms whereby calcium compounds flocculate clay, one in which the calcium compounds react with the core of the particle in a manner previously discussed, and which is primarily operative in neutral or acid systems, and another in which the calcium compounds react with the hydrophilous surface of the particles and which is mainly operative in alkaline systems. What has been called in these papers for purposes of preliminary discussion the "enhancement" of the flocculation of clay by calcium compounds brought about by the addition of alkali, must now be regarded not so much as a real enhancement of the same process but as the production of a different and more rapid process.

Some attention may now be given to the volume of the coagulum formed by flocculating clay with calcium compounds. The volume of coagula in general has been studied by Pickering<sup>1</sup> and is attributed by him to the formation of heavily hydrated compounds. In essential accordance with Pickering's view the volume of clay coagula formed by treatment with calcium compounds has been attributed by the writer to the formation of such complexes as the "hydrated silicate of calcium" which is formed by adding calcium hydroxide to colloidal silica. That precipitate has been studied by Le Chatelier (*loc. cit.*) and the analogy between it and the calcium hydroxide-clay coagulum has been set out by the present writer elsewhere<sup>2</sup>.

In elaboration of this theory of the volume of the precipitate formed by the combined action of calcium ions and hydroxyl ions on clay, it may be noticed in the first place that the volume (as well as the rate of flocculation) is greater when the hydroxyl ion is brought into contact with the clay first; that within certain limits it is greater the greater the time that elapses between the addition of the hydroxyl ions and the addition of the calcium ions; and that within certain limits it is greater the greater the amount of alkali used. These differences were not observable with suspensions of Kahlbaum silicic anhydride.

It is concluded from these facts that the hydroxyl ion may operate in two ways in its collaboration with the calcium ion in the flocculation of clay. First, as already discussed, it produces the alkaline reaction of

<sup>1</sup> Pickering, S. U., *Proc. Roy. Soc.* **94** (A), 1918.

<sup>2</sup> *Trans. Faraday Soc.* **17**, 1921.

the medium which is necessary for the combination of the calcium with, or the absorption of the calcium by, the emulsoid matter: second, when added *before* the calcium ions it disperses or peptizes the clay particles thereby producing a larger (emulsoid) surface and a corresponding increase of the first action. The action of the calcium ion is qualitatively the same whether added after or together with the hydroxyl ion, but if added after the hydroxyl ion the action is greater because there is a greater amount of one of the reacting substances (namely the surface colloids of the particles) which greater amount is produced during the time the hydroxyl ion is present without the calcium ion.

It is interesting and instructive to notice the effect of hydroxyl ions and calcium ions on suspensions containing in addition to clay a proportion of the larger particles. The experiments described show that a certain proportion of clay to silt can be obtained such that an alkaline suspension is flocculated by a calcium salt much more effectively during the first stages of flocculation and much less effectively during the later stages. Further, these critical mixtures show a more rapid flocculation when the calcium salt is added before the alkali than when the alkali is added first, which is the reverse of what takes place with suspensions of clay alone. The volume of the coagulum does not appear to be affected by the order in which the alkali and the calcium salt are added—only the rapidity of flocculation is influenced. These facts seem to be in accordance with the earlier facts of these papers. It follows from those earlier facts that if a calcium salt is added to an *alkaline* suspension of clay and silt the predominant tendency will be to flocculate the clay rather than the silt, and that if a calcium salt is added to a *neutral* suspension of clay and silt the predominant tendency will be to flocculate the silt rather than the clay. If the mixture contains clay in decided excess it will behave as clay throughout the flocculation process, and if it contains silt in excess it will behave as silt. But if it contains clay and silt in effectively equal amounts, then the superior flocculation of the clay in the alkaline suspension will reduce the clay surface, *i.e.* will reduce the effective proportion of clay to silt so that after some of the clay has been flocculated silt may dominate the system. This seems adequately to explain the fact that in such mixtures flocculation is, in its first stages, superior in alkaline media and later becomes inferior therein. Again, in the flocculation of such critical mixtures in *neutral* suspension the silt will be flocculated at a greater rate than the clay, and the effective proportion of clay to silt will be thereby increased. This appears to offer an explanation of the experimental fact that when

a neutral calcium salt has been added to such a suspension and an alkali is added subsequently, flocculation is enhanced and is superior to that induced when the alkali is added before the calcium salt. At the time of the subsequent addition of alkali sufficient of the silt has been flocculated to enable the clay to dominate the system which then behaves as clay.

*The influence of the hydroxyl ion upon the subsequent flocculation of clay by acids.*

Silica suspensions are not ordinarily flocculated by acid, but if the suspension is previously treated with ammonium hydroxide, the addition of an excess of acid produces flocculation. Similarly dilute silica sol after shaking with alkali is precipitated by acids. Clay suspensions are, of course, flocculated by acids, but the action is enormously increased by the previous addition of alkali. Here, as when calcium salts are used, it is thought that the action of acid *alone* is, directly or indirectly, an action between the acid and the core of the particle, and that the increase of the action of acid after treatment with alkali is mainly due to another mechanism involving the surface colloids.

*The relation of the core of the particle to its colloidal surface.*

Hitherto in these papers the terms "clay" and "silt" have been used rather crudely. For convenience the term clay has been used to describe the smallest particles and the term silt to describe slightly larger particles. It is now desirable to refer more carefully to the distinction and to point out that the different behaviour of the smallest and the slightly larger particles in a given soil is essentially a difference in specific surface. This seems to be shown by the fact that grinding the silt and thereby increasing its specific surface brings its behaviour to calcium hydroxide more nearly into line with that of clay. There is no reason to suppose that the surface of the clay particles is qualitatively different from the surface of the larger particles. It has been argued in these papers that clay behaves like an emulsoid colloid and silt like a suspensoid, but both large and small particles are regarded as being particles with a siliceous—or otherwise emulsoid—surface. The difference between the smallest and the slightly larger particles is that the gel surface of the smallest particles is very large relatively to the "core" of the particle and dominates the system whereas the surface of the larger particles is small relatively to the core of the particles and does not dominate the system.

Further, the distinction between the smallest particles which are so easily flocculated by calcium hydroxide and the larger particles which are not thus readily flocculated, is not likely to be *accurately* defined in terms of the diameter of the particle. If *all* soil particles *as particles* tend to be stabilized in suspension by the hydroxyl ion of calcium hydroxide, but have a surface which is coagulated by calcium hydroxide, the nett action of calcium hydroxide on any given soil particles will depend upon the ratio of the effective emulsoid-gel surface to the size of the particle. But the emulsoid surface may be differently developed in different soils according to the amount of attrition and weathering action to which the soil material has been subjected and consequently the upper limit of diameter of particles which are readily flocculated by calcium hydroxide may vary from one soil to another<sup>1</sup>. That upper limit of diameter may be extremely low in some soils which have not been subjected to much weathering: it is extremely low in certain Palaeozoic soils of North Wales as shown by experiments recorded in the earlier paper. Also, that upper limit may be very high in soils which by prolonged weathering or by the actual addition of emulsoid matter, have a highly developed emulsoid surface; for the "silt" of soils will flocculate like clay if treated with sufficient silica, and Hall and Russell<sup>2</sup> state that the difficulties of working fine silt soils largely disappear when large amounts of organic matter are present.

Hence, while it is convenient to speak of the difference between the behaviour of "clay" and the behaviour of "silt" towards calcium hydroxide, it is not suggested that any particular diameter (*e.g.* 0.002 mm.) always and rigidly defines the upper limit of the size of the particles which behave anomalously to calcium hydroxide, but that that upper limit will vary with variations in the ratio of emulsoid surface to particle.

*The effect of the concentration of a calcium hydroxide solution  
upon its action on clay.*

In the experiments described in the first paper in support of the theory that calcium hydroxide is, contrary to the usual teaching, a better flocculant of clay than a neutral calcium salt, the calcium hydroxide was generally added to the suspensions in such amounts that the final concentration was  $\frac{N}{75}$  or  $\frac{N}{100}$ . Experiments with lower concentrations are described in this paper and they show that in very low concentrations

<sup>1</sup> It will be clear that a *gel* surface may be great or small without material alteration in the diameter of the particle.

<sup>2</sup> Hall and Russell, "Soil Surveys and Soil Analysis," *Journ. Agric. Sci.* **4**, 1911.

calcium hydroxide is far inferior to a neutral calcium salt as a flocculant of clay. The action of calcium hydroxide in higher concentrations is clearly the action which follows the ordinary liming of clay soils, but it is desirable to consider the more complete facts, namely, that in very low concentrations calcium hydroxide has a "normal" action, the deflocculating action of the hydroxyl ion being apparent, whereas in higher concentrations its action, as interpreted by the ultimate result, is reversed. In the experiments described here the concentration below which the hydroxyl ion of calcium hydroxide deflocculates clay is of the order of  $\frac{N}{1000}$ . This deflocculating action may be due to a definite hydrogen ion concentration in the original suspensions, such that measurable amounts of alkali are added before the hydroxyl ion concentration becomes greater than  $10^{-7}$  mols. per litre. Or it may be that for any given clay suspension a definite concentration of calcium hydroxide must be reached before the action with the hydrophilous surface becomes dominant and apparent. It is only intended here to state the fact that very small concentrations of calcium hydroxide deflocculate clay while higher concentrations flocculate it as previously described and discussed. The action of these small concentrations of calcium hydroxide is being further investigated.

*The emulsoid surface of the soil particles.*

The emulsoid surface of the soil particles is doubtless complex. Silica may reasonably be supposed to form a prominent part of the complex, but the possible existence of other emulsoids must not be forgotten. The deep subsoil clay used in some of the experiments described above was chosen because of the absence of organic matter therein. Its behaviour in those experiments indicates that organic colloids *alone* are not to be held responsible for the behaviour of clay to calcium hydroxide. The behaviour of humus to calcium hydroxide is very similar to that of silica and the precipitation of "calcium humate" as well as of "calcium silicate" may contribute a part in the calcium hydroxide flocculation of clay. Nothing can be said here about the relative importance of different colloids in connection with the phenomena described in these papers. It is merely desired to say that the possibility of the action of colloids other than silica (which has been considered throughout as typical) has not been overlooked and some means of investigating the complexity of the soil colloids is being sought.

## SUMMARY.

Three types of mechanism whereby clay is flocculated by electrolytes, are discussed.

1. *Normal or direct flocculation* the mechanism of which is precisely that by which electrolytes coagulate electro-negative suspensoids. The isoelectric point theory of such flocculation is assumed in this paper. Experimental evidence is brought forward to show that iron and aluminium salts behave in this "normal" way.

2. *Indirect flocculation* which takes place whenever the electrolyte reacts with the clay and thereby brings into solution from the clay other substances which cause flocculation. The action of some neutral salts and of acids is regarded as being largely indirect.

3. *Abnormal flocculation* which is the result of a reaction between the added flocculant and the emulsoid surface of the clay particle. The action of calcium hydroxide is placed under this heading for reasons set forth in the earlier paper.

Because of its obvious bearing on important agricultural operations, the action of calcium hydroxide on clay is considered more fully. The following are the salient points of that consideration:

(i) Although it is reversible to carbonic acid the flocculation of clay by calcium hydroxide is not due to the formation of calcium carbonate. Several experimental results are set forth to show this, the chief is that barium and calcium hydroxides behave quite differently to carbonate and carbonic acid, and alike to clay.

(ii) The action of calcium hydroxide on clay is an action on an "emulsoid gel surface" and not an action on a sol present in gross amount.

(iii) The hydroxyl ion may perform at least two functions in its co-operation with the calcium ion in the flocculation of clay: (a) when added *with or after* the calcium ion it produces the alkalinity necessary for the reaction between calcium compounds and silica, etc., as discussed in the earlier paper; (b) when added *before* the calcium ion it not only produces this alkalinity but also peptizes the clay with the production of a greater amount of the emulsoid surface and a consequent increase in the rapidity of flocculation and the volume of the coagulum.

(iv) Critical mixtures of clay and silt—in which neither wholly dominates the system—can be obtained by decantation at an appropriate time during the sedimentation of soil suspensions. It is shown experimentally that when the clay of such mixtures is flocculated more rapidly

than the silt, the silt then becomes dominant; and that when the silt is flocculated more rapidly than the clay, the clay then becomes dominant.

(v) The different behaviour of "clay" and of "silt" to calcium hydroxide is not regarded as due to any *essential* difference in the structure of these particles but as being determined by the ratio of the emulsoid surface to the core of the particle. In clay the surface dominates the system, in silt the core dominates the system.

(vi) In *very low* concentrations the hydroxyl ion appears to behave normally and to militate against the flocculation of clay by calcium salts.

(vii) Organic emulsoids may, in ordinary soils, be partly responsible for the anomalous action of calcium hydroxide on clay, but it is shown that calcium hydroxide exhibits that anomalous action on a deep subsoil clay containing no organic matter.

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